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Br J Vener Dis 1982;58:211-9

Scanning electron microscopy of the attachment of Treponema pallidum to nerve cells in vitro

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SUMMARY *Treponema pallidum* (Nichols strain) was incubated with cultured nerve cells derived from rat embryos. Primary cultures were established from dorsal root ganglia, superior cervical ganglia, and spinal cord. Using phase contrast microscopy treponemes were seen to interact with the nerve cells in a similar manner to other cultured mammalian cells. Organisms began to attach within minutes after inoculation, actively motile organisms attached at the tip of one end, higher numbers of organisms attached with continued incubation, and attached organisms survived longer than unattached organisms. *T pallidum* attached both to nerve cell bodies and to neuronal processes of each of the three nerve cell cultures. As shown by scanning electron microscopy the mechanism of attachment was identical to that of cultured cells derived from rabbits testis, rat skeletal muscle, and human cervical carcinoma. There was no indentation or swelling of the cultured cell surface at the point of attachment, just a close physical proximity of organisms and cells. These techniques provide a biological means of studying the in-vitro detrimental influences of micro-organisms on nerve tissue.

Introduction

Clinical manifestations of tertiary and congenital syphilis frequently result from treponemal damage to the central nervous system. Parenchymatous neurosyphilis is characterised by pronounced destruction of the cerebral cortex, dorsal nerve roots, and spinal cord. Three reports have indicated that *Treponema pallidum* is also present within nerve tissue in primary syphilis, although nerve damage is not apparent. Gregoriew[1] demonstrated treponemes in the endoneurium and perineurium of nerve fibres, and Ovcinnikov and Delektorskij[2] observed treponemes in various parts of nerve fibres. Wrzolkowa and Kozakiewicz[3] showed slight degeneration of axon terminals in lesion material; treponemes, however, were not observed in this tissue.

T pallidum attaches to numerous types of cultured mammalian cells.[4 5] This attachment appears to be related to pathogenesis,[6] and similar attachment to host tissues in vivo may be critical in initiating disease.[7 8] To this point, treponemes have been shown to attach to at least 40 different types of cultured cells derived from testis, skin, cervix, kidney, spleen, lung, urethra, lymph node, cornea, liver, prepuce, nose, footpad, and ear.[9] This is in accordance with the syphilitic disease process in which *T pallidum* infects most tissues of the body.

The in-vitro interaction of treponemes with cultured cells derived from nerve tissue has not been studied. The organisms do attach to a C-6 cell line derived from rat glioma.[10] Glial cells, however, are non-neuronal cells that provide structural support but do not transmit nerve impulses. Recently, techniques have been reported for culturing neuronal cells from embryonic tissues. To our knowledge, there is only one report of the in-vitro interaction of micro-organisms with nerve cells; herpes simplex virus damaged cells derived from dorsal root ganglia.[11] The purpose of this study was to demonstrate the attachment of *T pallidum* to three different types of nerve cells.

Materials and methods

T PALLIDUM

The Nichols strain was maintained by intratesticular passage in rabbits. Each testis was inoculated with 1×10^7 to 3×10^7 treponemes. After a satisfactory

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Accepted for publication 17 January 1982

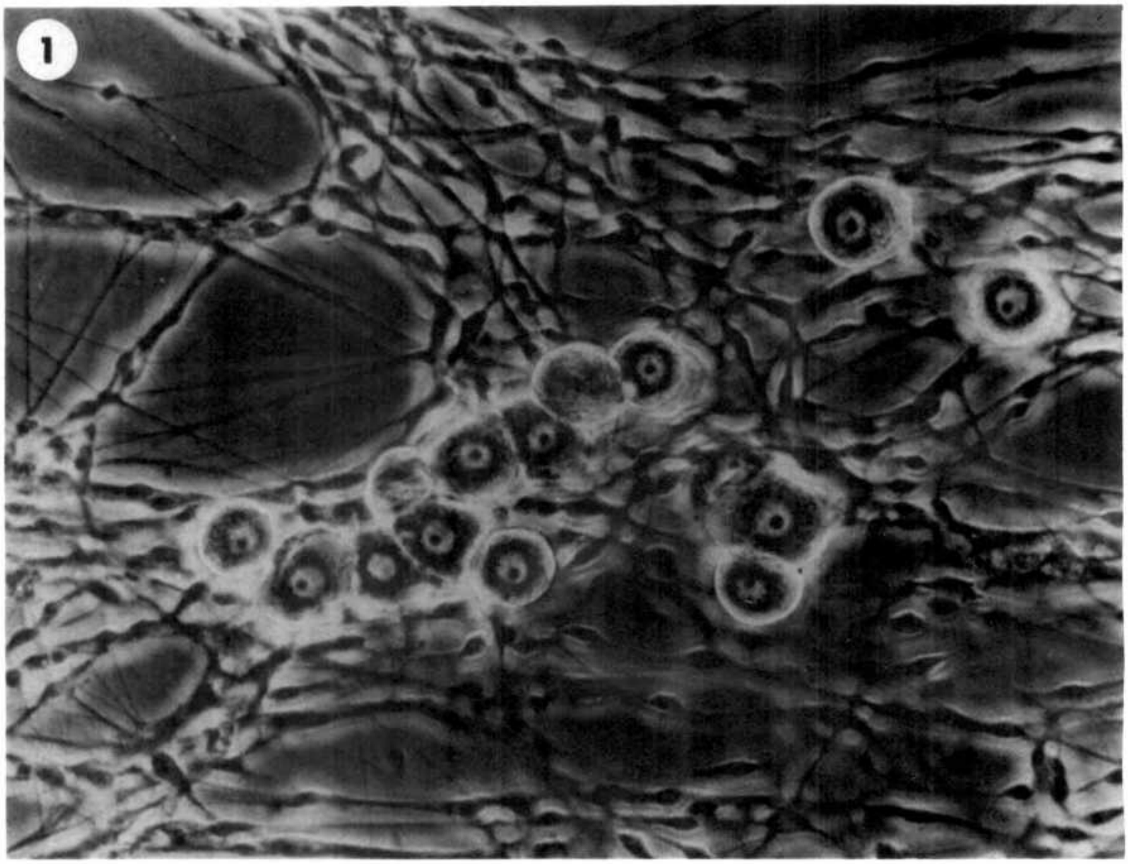

FIG 1 *A phase contrast micrograph of a portion of a nerve cell culture sheet from dorsal root ganglia. The nerve cells grew on top of the background fibroblasts and were rounded with prominent nuclear and nucleolar regions. (× 1400)*

orchitis had developed in 9-12 days animals were killed and the testes removed, minced with scissors, and extracted at room temperature in tissue culture medium. This medium contained Eagle's minimal essential medium supplemented with 4 mmol/l $NaHCO_3$, 30 mmol/l HEPES (N-2-hydroxyethylpiperazine N-2 ethanesulphonic acid), and 10% heated normal rabbit serum (56°C for 30 minutes). After 10-20 minutes the preparation was centrifuged at 1000 × *g* for 10 minutes to sediment particulate matter. This was followed by high-speed centrifugation at 17 000 × *g* for 30 minutes at 4°C. The pelleted treponemes were then resuspended in fresh medium at 3×10^7 to 6×10^7 organisms/ml.

NERVE CELLS

Preparations were made of dorsal root ganglia, superior cervical ganglia, and spinal cords according to the methods of Ransom *et al.*[12] The dorsal root ganglia were prepared from 17-19-day-old rat embryos (Sprague-Dawley). Dissected ganglia were placed into 5 ml of 0·25% trypsin in Hank's balanced salt solution minus calcium and magnesium. After 30 to 45 minutes at 37°C, the trypsinised cells were washed once and resuspended in Eagle's minimal essential medium supplemented with 600 mg/100 ml glucose, 2% chick embryo extract, 10% heated horse serum (56°C for 30 minutes), 10% fetal bovine serum, and gentamicin at 50 μg/ml. A volume of 0·5 ml of medium was used per embryo. Five drops of this cell suspension were added to 35 × 10 mm culture dishes coated with calf skin collagen. Cultures were incubated at 37°C in 95% air and 5% carbon dioxide. After 24 hours, 1·5 ml medium was added and incubation continued. After the cultures had almost reached confluency, medium containing 10 mmol/l cytosine arabinoside without fetal bovine serum was added for 48 hours to decrease the amount of fibroblastic

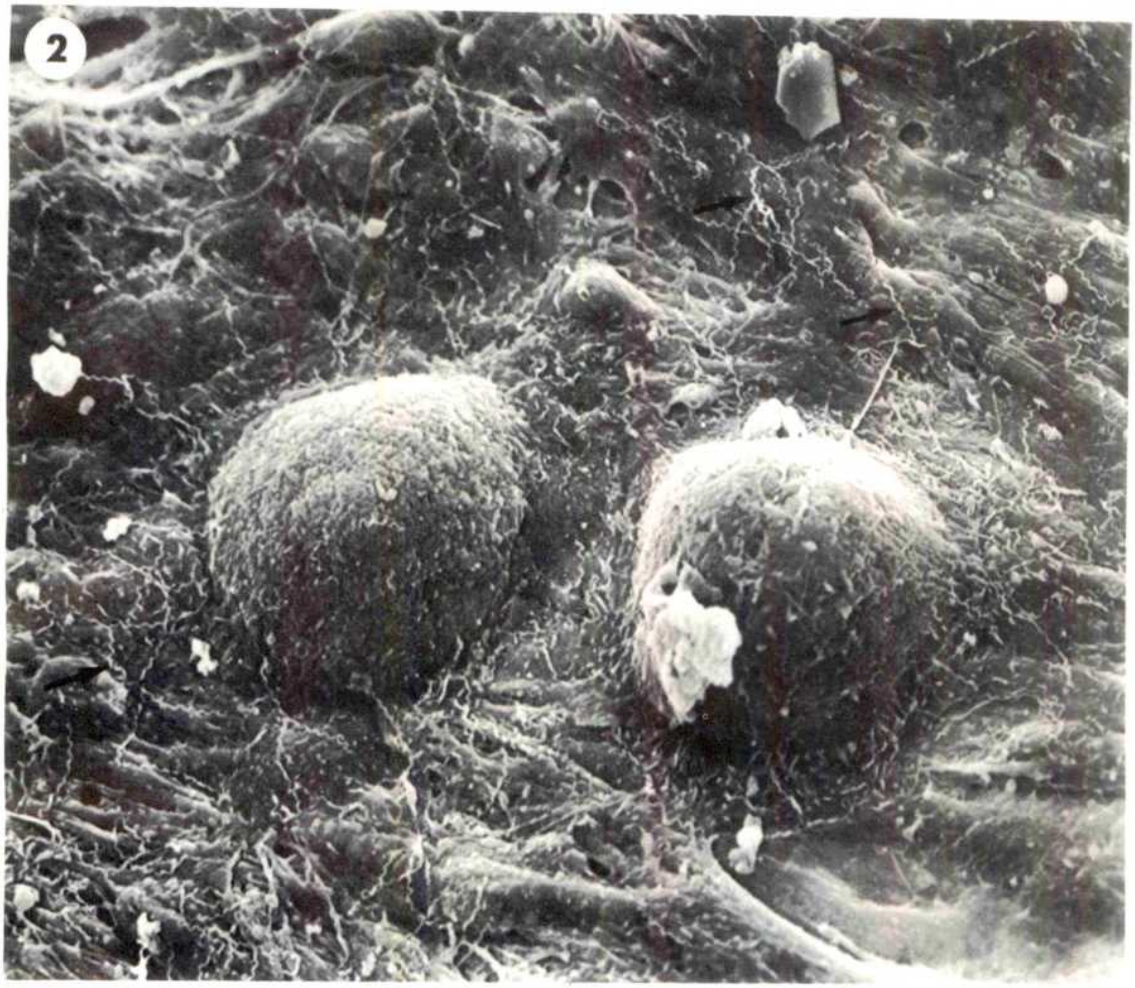

FIG 2 *A scanning electron micrograph of a portion of a nerve cell culture sheet from dorsal root ganglia. Two nerve cells were raised and grew over the flattened fibroblasts in the background. Greater numbers of treponemes (arrows) were attached to the fibroblasts. (× 1800)*

cell background. Cytosine arabinoside was then removed and culture medium replaced. These cultures were maintained for 4-6 weeks; medium was changed twice weekly.

Cultures of superior cervical ganglia were prepared from 21-day-old rat embryos. Conditions and laboratory manipulations were similar to those described above. The culture medium was additionally supplemented with 20 μg/ml 7S nerve growth factor (Collaborative Research, Waltham, Massachusetts). A volume of 0·25 ml medium was used per embryo.

Cultures of spinal cord were prepared from 13-14-day-old rat embryos. Meningeal coverings and spinal ganglia were removed and spinal cord tissue cut into 2-mm fragments. Additional procedures were similar to those described for preparation of the cultures of dorsal root ganglia.

TREPONEMAL INOCULATION

Nerve culture cells were gently washed three times with 2 ml treponemal medium. Cultures were then inoculated with 2 ml treponemes and incubated at 37°C in an atmosphere of 2·5% oxygen, 5% carbon dioxide, and 92·5% nitrogen.

SCANNING ELECTRON MICROSCOPY

Cultures were rinsed twice in Hank's balanced salt solution and fixed in situ with 3% glutaraldehyde in 0·1 mol/l sodium cacodylate buffer (pH 7·35) at room temperature for one hour. After a thorough

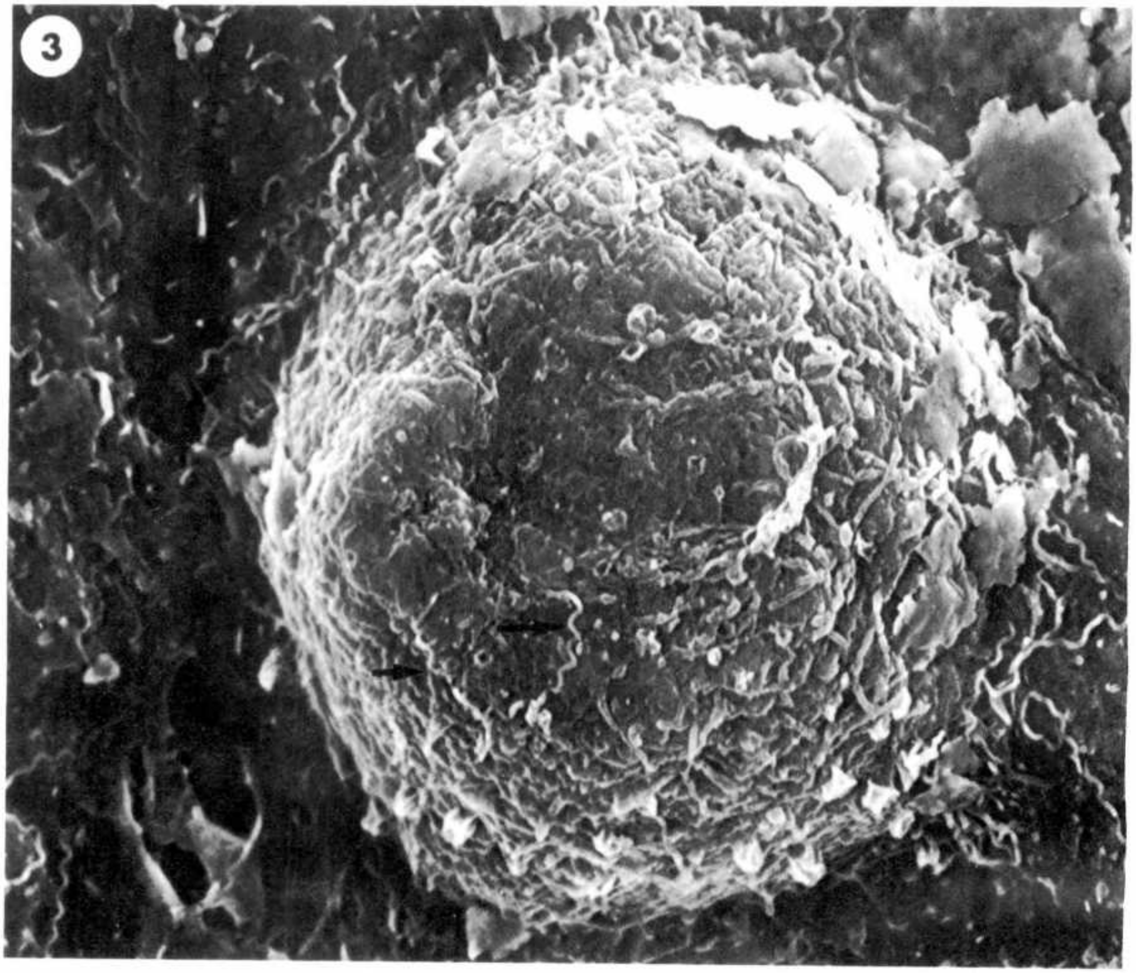

FIG 3 *Treponemes (arrows) attached to nerve cells derived from dorsal root ganglia after 45 minutes' incubation. (× 3800)*

rinsing with 0·1 mol/l sodium cacodylate buffer containing 7·5% sucrose, the cultures were post-fixed in 1% OsO_4 with 0·1 mol/l sodium cacodylate for 30 minutes, washed three times in double distilled water, stained in 2% uranyl acetate, and dehydrated in aqueous grades of alcohol to 100%. The samples were critical-point dried using CO_2, mounted on aluminium stubs with double-stick tape, and coated with gold-palladium using a Hummer II evaporator. Observations were made using an AMR 100 scanning electron microscope.

Results

In the initial studies phase contrast microscopy was used to characterise treponemal interaction with cultured nerve cells. The neuronal cells can be presumptively identified morphologically. Fig 1 shows neuronal cells as well as background fibroblasts in a primary culture derived from dorsal root ganglia. The larger, rounded, raised neuronal cells characteristically grew on top of the monolayer of fibroblasts and showed a well-defined nucleolus. The fibroblasts which were below the field of focus showed typical fibroblastic, flat, elongated morphology.

The treponemes attached to the neuronal and fibroblastic cells. Attachment was identical to that of other cultured mammalian cells.[4 6 8 10 13 14] Attached organisms retained the typical motility of bending, flexing, and rapidly rotating about their axis without moving from their site of attachment. Attachment was usually mediated through the tip of one end of the treponeme. Occasionally a few organisms attached at both ends retaining active motility of the

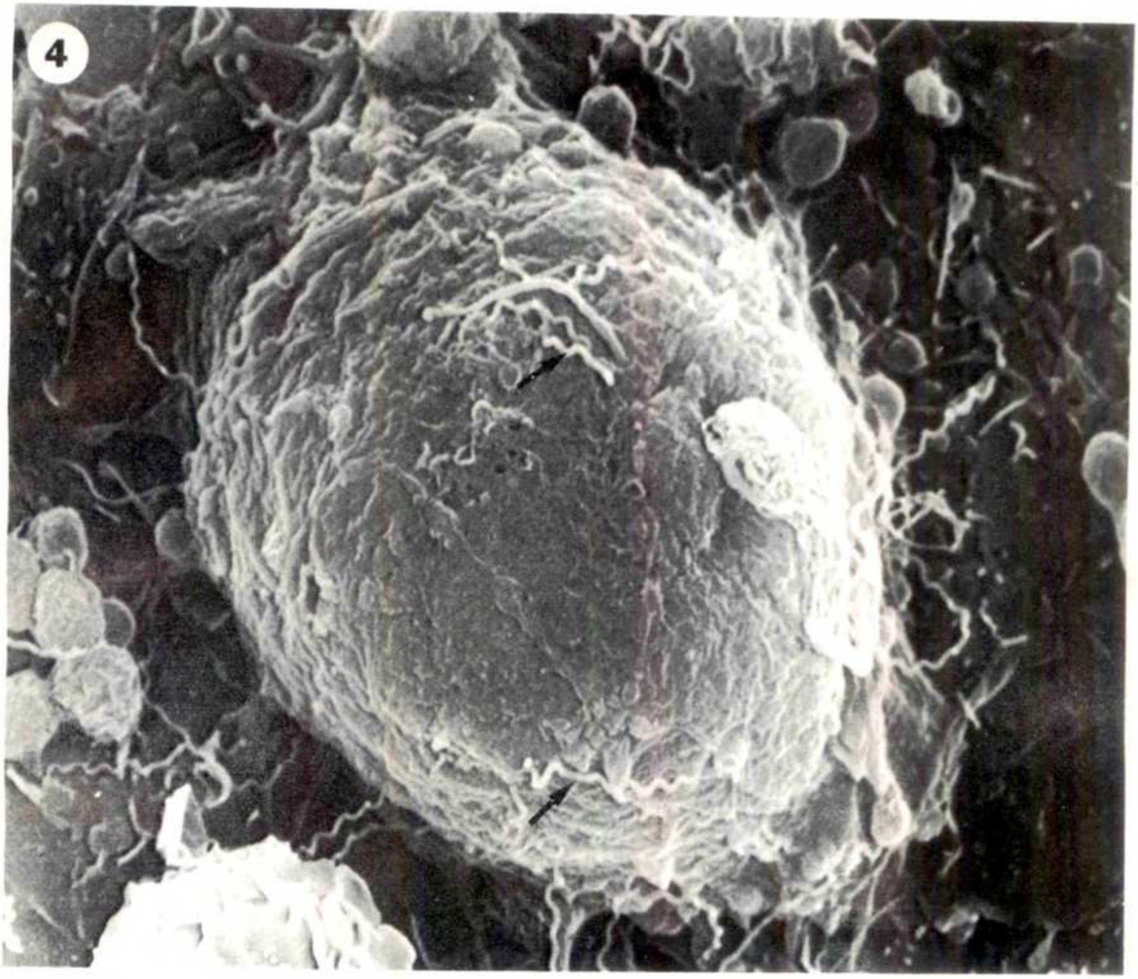

FIG 4 *Treponemes (arrows) attached to nerve cells derived from spinal cord. These organisms were also present on the background fibroblasts. Cells were incubated with the organisms for 45 minutes. (× 4500)*

middle portion of the organism. Extensive washing of the cultures effectively removed unattached but not attached *T pallidum*. Organisms began to attach immediately after inoculation, and with increasing periods of incubation higher numbers of treponemes were attached to each cell. In agreement with other studies, attached organisms retained active motility at least 24 hours longer than unattached organisms; also, heat-inactivated treponemes failed to attach, indicating that attachment is not due to passive sticking or to phagocytic-like activity of the cultured cells. The above findings with cells from dorsal root ganglia were also observed with cells from superior cervical ganglia or spinal cord.

Scanning electron microscopy provided a three-dimensional view of treponemal attachment to the nerve cells. *T pallidum* was added to culture dishes containing cells derived from dorsal root ganglia, superior cervical ganglia, or spinal cord. After incubation for 45 minutes the cultured cells were washed three times with medium to remove unattached treponemes. Fig 2 shows a micrograph at low magnification of cultured cells from dorsal root ganglia. The two nerve cells are readily identified by their rounded raised appearance and by their growth on top of the background monolayer of fibroblasts. Attached treponemes were difficult to see at this low magnification.

Higher magnifications are shown in figs 3-5. All treponemes present were attached; the washing procedures effectively removed unattached treponemes. *T pallidum* attached to nerve cells from dorsal root ganglia (fig 3) and from spinal cord (fig 4). Larger numbers of organisms attached to nerve cells from

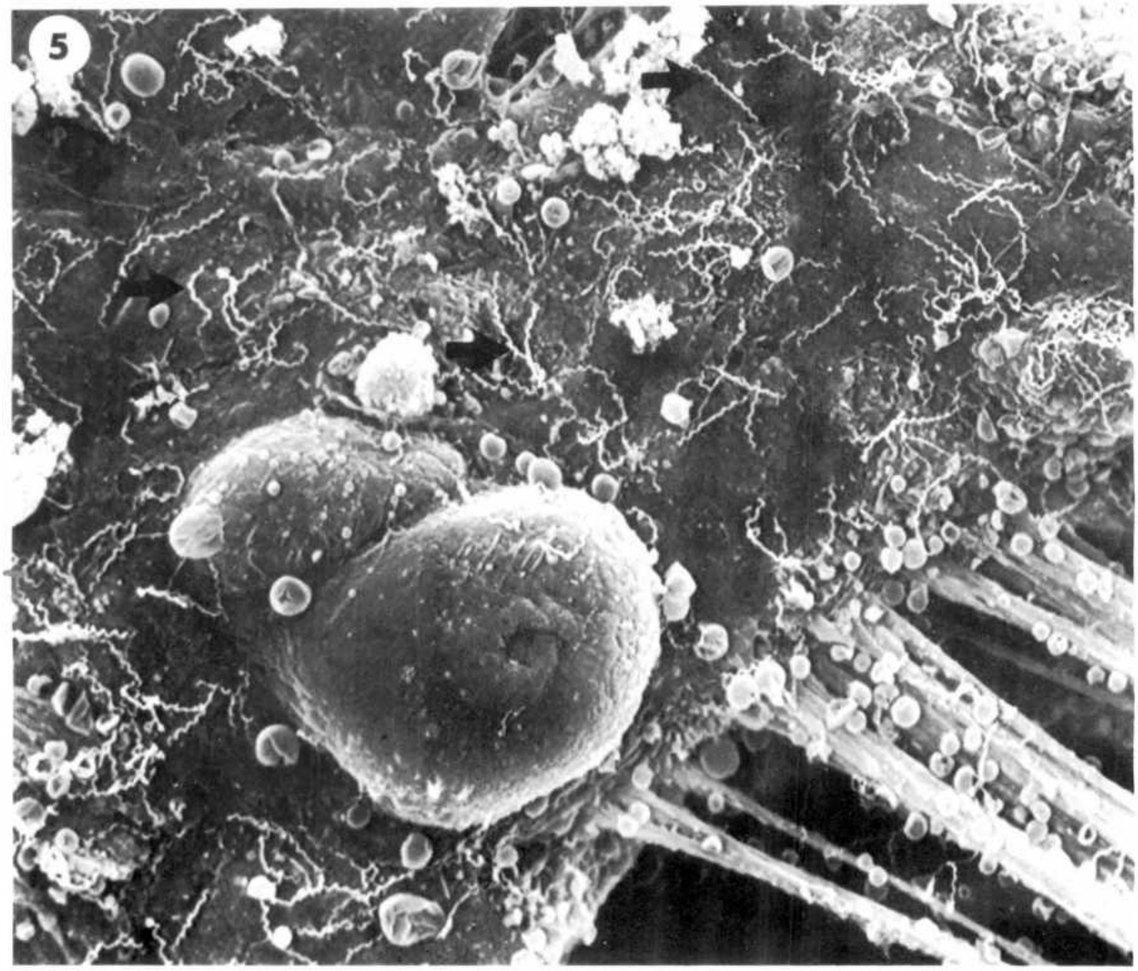

FIG 5 *Numerous treponemes (arrows) attached to the surface of a portion of a nerve cell derived from superior cervical ganglia after 45 minutes' incubation. (× 1700)*

superior cervical ganglia. Fig 5 shows only a small portion of one of these cells which have a much larger diameter (100 μm) than the other two nerve cell types (30 μm). In these micrographs the organisms appeared to attach along their entire length. This was artifactual, however, as shown by phase contrast microscopy of actively motile treponemes. *T pallidum* attached only at its very tip. During fixation the organisms settled on to the cultured cell surface giving the appearance of full length attachment rather than tip attachment.

Besides attaching to the nerve cell body *T pallidum* also attached to the neuritic processes that extend outward from the nerve cells. Fig 6 shows treponemes attached to a neuronal process emanating from a cell derived from superior cervical ganglia. Organisms were attached along the full length of the process. Identical findings were observed with neuritic processes of cells derived from dorsal root ganglia and spinal cord.

In figs 3-6 nerve cells were incubated with treponemes for 45 minutes. With further incubation increased numbers of organisms attached to each of the three nerve cell types. Fig 7 shows a portion of a dorsal root ganglia cell after incubation with treponemes for six hours; note the larger number of attached organisms relative to the cell shown in fig 3.

Discussion

Syphilitic infection frequently affects nerve tissues. In the primary stage treponemes have been seen

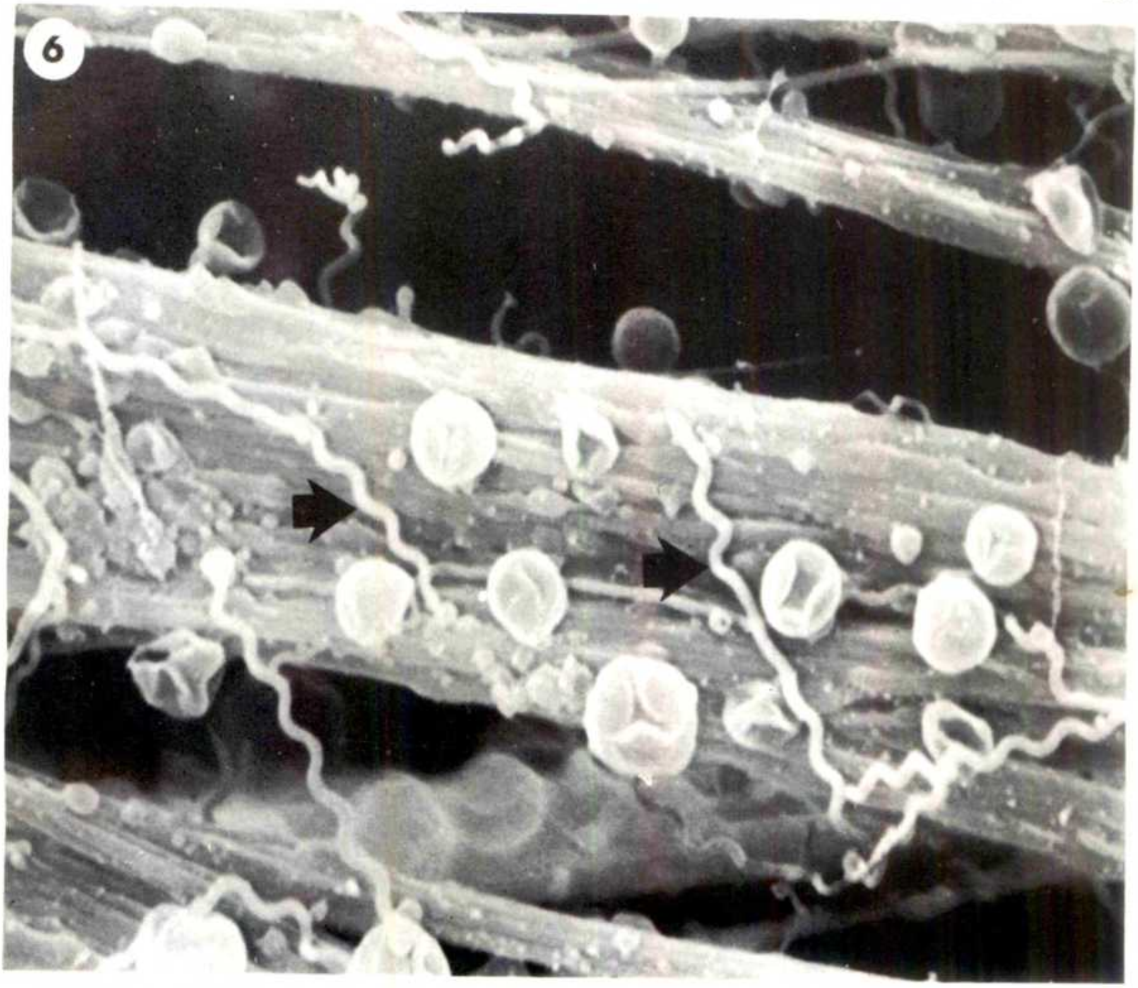

FIG 6 *Higher magnification of treponemes associated with neuritic processes extending from a cell derived from superior cervical ganglia. Arrows indicate organisms. (× 9000)*

within nerve fibres of lesion material[1 2]; in addition, some axon degeneration has been reported.[3] In both tertiary and congenital syphilis nerve destruction is readily apparent. Clinical manifestations result from pronounced degeneration of the cerebral cortex, dorsal nerve roots, and spinal cord. The specific mechanisms of treponemal-mediated damage to nerve tissue are unknown. Tissue culture has provided a valuable means of elucidating some aspects of the pathogenesis and immunology of *T pallidum*.[7] It has been suggested that attachment of treponemes to host tissues is a critical part of the disease process.[4 6 7] The unique in-vitro system described in this paper should provide useful information about potential in-vivo influences of *T pallidum* on specific nerve tissues.

Our findings represent the first demonstration of the in-vitro interaction of bacteria with cultured nerve cells. *T pallidum* attached to nerve cell bodies and to neuritic extensions of cells derived from dorsal root ganglia, superior cervical ganglia, and spinal cord. This attachment is quite similar to that described for other cultured mammalian cells.[4 5 8 10 13 14] Organisms began to attach within minutes after inoculation, and with continued incubation higher numbers of treponemes attached. The cultured cells prolonged treponemal survival as shown by the better retention of motility of attached compared with unattached treponemes. In addition, heat-inactivated *T pallidum* failed to attach. Phase contrast microscopy of viable preparations showed that attachment involved the tip of the organisms interacting

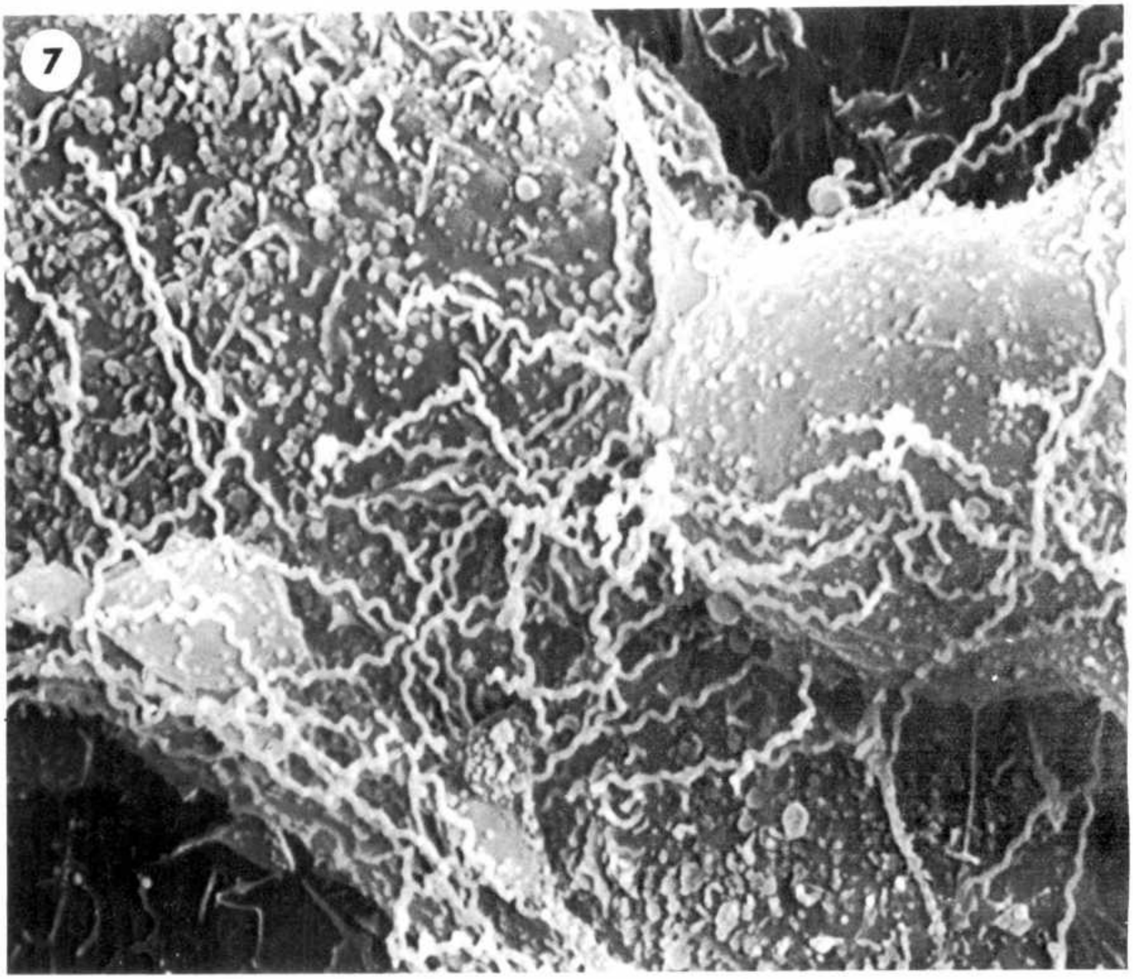

FIG 7 *After six hours' incubation treponemes formed a dense network on the surface of a portion of a nerve cell derived from dorsal root ganglia. (× 5900)*

with the nerve cell surface. Attached organisms retained characteristic motility of bending, flexing, and rotating without moving from the point of attachment. Scanning electron microscopy of fixed preparations showed that there was no indentation or swelling of nerve surface topography at the point of attachment. This confirms previous scanning electron microscopy of treponemal attachment to cultured cells derived from rabbit testis, rat skeletal muscle, and human cervical carcinoma.[8 15 16]

Because appropriate techniques are available, tissues derived from embryonic rats were used. Syphilitic infection of rabbits mimics infection of humans; in contrast, infection of rats results in minimal tissue response.[17] Overt clinical lesions usually do not occur and it is difficult to detect organisms in tissues. Treponemes in asymptomatic infection can be shown by transfer of lymph node material to rabbits, who subsequently develop syphilitic lesions.[17] The interaction of *T pallidum* with cultured cells derived from rabbits is comparable to the interaction with cultured cells derived from species that show minimal syphilitic infection such as rat, mouse, dog, monkey, and pig.[6 13 14] It is likely that the basis for minimal infection in these animals is not attributed to innate differences within tissues. More probably, the lack of symptomatic infection reflects differences in either serum factors or in more effective humoral and cell-mediated immune responses.[9] Thus, relevant insights into the pathogenesis of *T pallidum* should result from these studies using nerve tissue derived from rats.

We would like to acknowledge the excellent technical assistance of Paul Lima for tissue culture preparations, Eileen Gannon for treponemal preparations, and Anne Utyro for developing and processing the photomicrographs. This research was supported by the Public Health Service and National Institute of Allergy and Infectious Diseases grant AI 16-585, the National Institute of Neurological and Communicative Disorders and Stroke grant NS-13325, and the Minnesota Medical Foundation grant 0762-5836.

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Electrophysiological dysfunction and cellular disruption of sensory neurones during incubation with Treponema pallidum

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SUMMARY *Treponema pallidum* (Nichols strain) was incubated with cultured nerve cells derived from dorsal root ganglia of rat embryos. The electrophysiological response of these neuronal cells was then investigated. Cells exposed to 2×10^8 treponemes/ml responded abnormally after 13 hours and failed to respond after 18 hours. In contrast, control preparations exposed to heat-inactivated treponemes or to culture medium responded normally after 72 hours. Extended incubation with viable treponemes resulted in various degrees of nerve cell disruption as shown by scanning electron microscopy. With some cells holes in the cytoplasmic membrane were detected; with others a coagulated matrix of apparent nuclear material and remnants of cytoskeletal elements indicated more severe destruction. These findings may explain the painless nature of many of the clinical manifestations of syphilis as well as the severe damage to central nervous system tissue in tertiary and congenital syphilis.

Introduction

Ovcinnikov and Delektorskij[1] demonstrated treponemal association with nerve cell tissues within material from syphilitic lesions. Although they did not observe nerve histopathology, they suggested that *Treponema pallidum* might inactivate nerve function; this in turn could explain the painless nature of most of the clinical manifestations of syphilis. The accompanying paper[2] described the interaction of *T pallidum* with cultured cells derived from dorsal root ganglia, superior cervical ganglia, and spinal cord. After six hours' incubation numerous treponemes were attached and the nerve cell morphology was not altered. The purpose of this study was to show that with extended incubation treponemes initially cause electrophysiological dysfunction then morphological disruption of dorsal root ganglia neurones in culture.

Materials and methods

T PALLIDUM

The Nichols strain was maintained by intratesticular passage. Conditions for extraction have been described.[2] After slow-speed centrifugation organisms were centrifuged at high speed ($18\,000 \times g$ for 30 minutes at 4°C) to remove the inflamed testicular constituents. The only departure from previous conditions was the omission of glutathione, cysteine, and dithiothreitol from the treponemal suspending medium. For inactivation by heat treponemes were placed at 56°C for 10 minutes.

NERVE TISSUE CULTURE CELLS

Nerve cells were isolated from dorsal root ganglia of 17-19-day-old rat embryos. Cell preparation, medium, and conditions of incubation have been described.[2] After the cells had grown for 3-4 weeks culture medium was removed and the cells were washed three times with 2 ml treponemal suspending medium. Different concentrations of treponemes were then added in a 2-ml volume. Cells were incubated at 37°C in an atmosphere of 92·5% nitrogen, 5% carbon dioxide, and 2·5% oxygen.

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Accepted for publication 15 February 1982

SCANNING ELECTRON MICROSCOPY

The techniques and fixation procedures for examining nerve cells using scanning electron microscopy have been described.[2]

ELECTROPHYSIOLOGICAL METHODS

Electrical recordings were made directly in the culture dishes on the stage of a Zeiss inverted microscope. The tip of a microelectrode was inserted into a nerve cell. Voltage differences were then measured between this intracellular electrode and a second electrode used as a ground immersed in the extracellular fluid. Electrical pulses were passed through the intracellular electrode and the change in voltage across the cell membranes monitored. Microelectrodes were positioned with a Leitz micromanipulator under direct observation at a magnification of × 400. The temperature, monitored by a thermistor, was maintained at 37°C with an overhead heat lamp. Recordings were made in tissue culture medium to avoid disturbances in the infective process and to promote cell survival over an extended period of time.

Fine-tipped glass microelectrodes were filled with 4 mol/l potassium acetate (pH 7·0) with resistances of 30-80 mΩ. A bridge circuit (Mentor N-950, Minneapolis, Minnesota) was used for simultaneous current stimulation and voltage recordings. The cells were stimulated with a depolarising pulse, and the action potentials were monitored on an oscilloscope and recorded on a Hewlett-Packard FM tape recorder. The electrophysiological responses were analysed with a computer programme written for a PDP-12 computer (unpublished data). Values for the different experimental conditions were compared using a standard statistical package.

Results

Neuronal cells were presumptively identified in the previous study[2] by their characteristic morphology. Definitive identification is based on the ability of these cells to respond to electrical stimulation with an action potential. Fig 1a shows a typical action potential and defines the indices of resting membrane potential, spike amplitude, maximum rate of rise, and after-hyperpolarisation maximum. Normal action potentials are readily distinguished from abnormal action potentials.

Attempts were made to evaluate the influences of *T pallidum* on the genesis and character of the action potential of cultured nerve cells. In preliminary experiments control preparations failed to respond appropriately. Analysis of different components of the culture medium showed that glutathione, cysteine, and dithiothreitol prevented typical responses. These three reducing agents were then omitted from the treponemal suspending medium. Various concentrations of treponemes were added to the cultured cells. Controls included culture medium alone or heat-inactivated treponemes. At different times after inoculation neuronal cells were electrically stimulated and the action potentials measured. In the first two experiments the two control preparations showed normal responses after incubation for 72 hours. In contrast, neuronal cells exposed to 2 × 10^8 treponemes/ml lost their ability to respond after eight hours in one experiment and after 10 hours in the second.

The results of a third experiment are shown in table I. Normal responses were defined as those that fell within ± 1 SD of the resting membrane potential and spike amplitude for the controls not containing

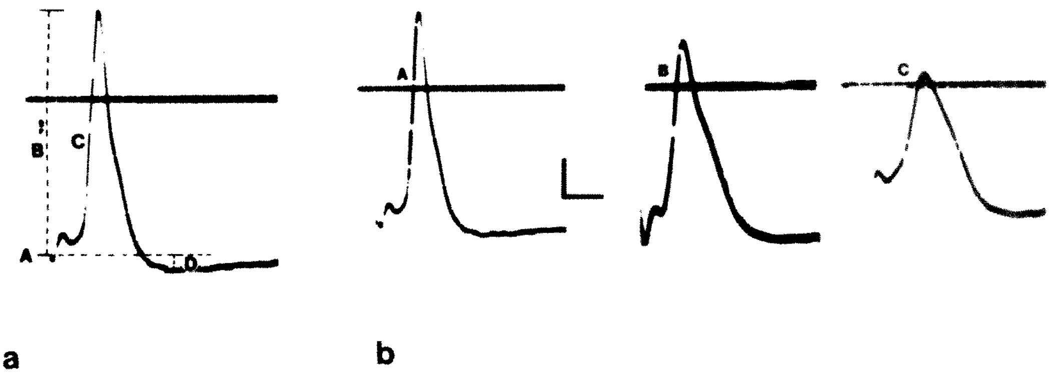

FIG 1(*a*) *A typical action potential of a dorsal root ganglion neurone. Electrophysiological indices are defined: A, resting membrane potential (mV); B, spike amplitude (mV); C, maximum rate of rise (mV/ms); D, after-hyperpolarisation maximum (mV). (b) Electrophysiological responses of neuronal cells exposed to culture medium or treponemes (A, normal control; B and C, abnormal treponemal-impaired). The calibration scale is 20 mV along the y axis and 1 ms along the x axis.*

TABLE I *Treponemal interference with electrophysiological responses of cultured dorsal root ganglia*

	Normal response/total tested*		
Time (hours)	*Without treponemes*	*2×10^8 Treponemes/ml*	*1×10^8 Treponemes/ml*
0	5/5	5/5	
5		5/5	
7		5/5	
10		5/5	
13		3/11	5/5
18		0/5	6/6
26	10/10	0/5	10/12

*Defined as those that fall within ± 1 SD of the resting membrane potential and spike amplitude for the controls not containing treponemes.

treponemes. At 0 hours the nerve cells exposed to control medium and to the two different concentrations of *T pallidum* functioned normally. After incubation for 26 hours, 10 of 10 cells in the control preparation responded normally. With treponemes at 2×10^8 organisms/ml abnormal responses were detected after 13 hours; only three of 11 cells responded normally. After 18 hours action potentials could not be elicited. With treponemes at 1×10^8 organisms/ml normal responses were detected in five of five cells after 18 hours and in 10 of 12 cells after 26 hours.

Fig 1b shows representative action potentials of control cells (A) and test cells (B and C) incubated with treponemes for 13 hours. The mean values determined by combining the indices for individual nerve action potentials in each experimental condition are shown in table II. For control preparations not exposed to *T pallidum* no significant differences were apparent after 0 and 26 hours; these two sets of values were combined. Incubation for 13 hours with 2×10^8 organisms/ml resulted in abnormal electrophysiology in eight of 11 neuronal cells. Comparison of the individual indices for these cells showed significant differences in resting membrane potential, spike amplitude, maximum rate of rise, and after-hyperpolarisation maximum compared with the controls. At this time the morphology of the neuronal cells was unchanged.

In further experiments nerve cultured cells were inoculated with 1 to 2×10^8 organisms/ml and controls of culture medium, heated treponemes, and the high-speed supernatant. Cultures were examined with phase-contrast microscopy. After incubation for 24 hours pronounced damage to both neuronal cells and fibroblastic cells was apparent in cultures exposed to viable treponemes. Neuronal morphology was not altered in the control preparations incubated for 24 hours.

Degradation was related to the concentration of *T pallidum*. Below 7×10^7 organisms/ml no morphological or electrophysiological changes were observed after 48 hours' incubation. After 48 hours, motility of the organisms was sharply decreased probably because of the omission of reducing agents from the culture medium.

Treponemal degradation of neuronal cells was evident by scanning electron microscopy. Cultures were inoculated with 2×10^8 organisms/ml. Figure 2 shows atypical morphology of a nerve cell after 16 hours' incubation. In contrast to the round elevated appearance of normal neurones the cells assumed a flattened morphology, and their surfaces were marked with irregular holes. Some cells were more damaged than others.

Various stages of treponemal-mediated nerve-cell degradation are shown in figs 3-5. Fig 3 shows the ghost-like remnant of an apparent dorsal root ganglia cell. The morphology was greatly altered. The nuclear profile in the centre of the cell deteriorated to a coagulated matrix. Remnants of cellular substratum attachment processes can be seen peripherally. At higher magnification of this peripheral area (fig 4) the edge of the cell is demarcated by fibrous extensions, which are associated with small bleb-like particles. These may

TABLE II *Selected indices of electrical properties of cultured dorsal root ganglia infected with treponemes*

		Electrical properties (mean ± SEM)			
*Preparation**	*Incubation time (hours)*	*Resting membrane potential (mV)*	*Spike amplitude (mV)*	*Maximum rate of rise (mV/ms)*	*After-hyperpolarisation maximum (mV)*
Without treponemes (15)	26	−55·61 ± 3·85	81·65 ± 9·13	204·11 ± 40·41	−6·40 ± 1·48
With 2×10^8 treponemes/ml (4)	7	−54·17 ± 2·40	89·18 ± 3·39†	220·60 ± 9·25	−3·65 ± 1·02†
(5)	10	−54·31 ± 1·25	83·79 ± 5·56	212·34 ± 26·2	−4·42 ± 1·18†
(8)	13	−50·12 ± 3·86†	65·66 ± 16·11†	146·64 ± 61·96†	−2·81 ± 0·9†
With 1×10^8 treponemes/ml (5)	13	−56·43 ± 3·45	77·11 ± 8·84	195·40 ± 38·73	−4·06 ± 2·11
(6)	18	−54·77 ± 4·50	80·51 ± 14·96	197·34 ± 63·51	−6·05 ± 2·02
(9)	26	−50·63 ± 4·29†	73·36 ± 14·16	170·52 ± 60·55	−5·8 ± 1·73

SEM = Standard error of mean
*No of neuronal cells tested given in parentheses
†Statistically significant, $P<0·05$

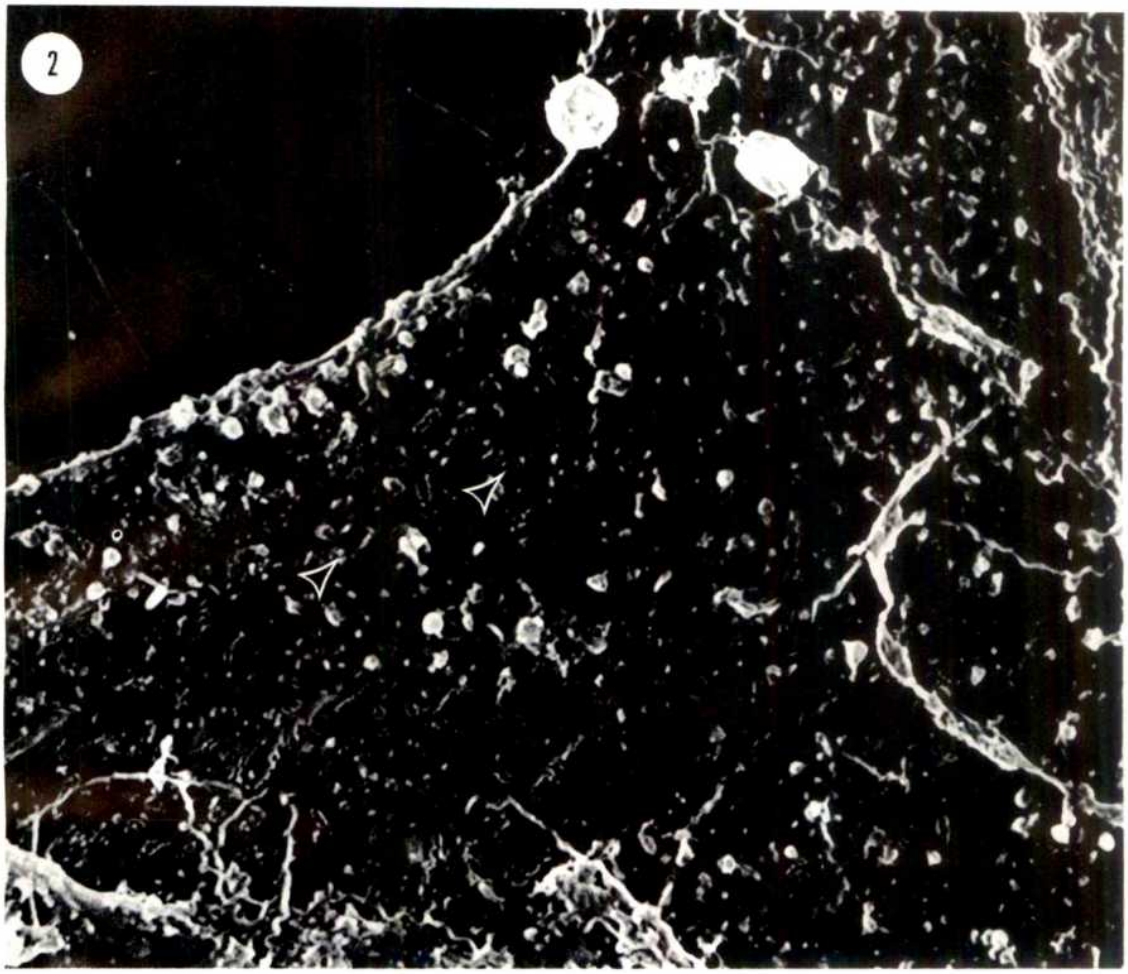

FIG 2 *Scanning electron micrograph of a nerve cell derived from dorsal root ganglia incubated for 16 hours with T pallidum. The flattened cell shows irregular holes (arrowheads) on its surface. (× 3900).*

represent the cytoskeletal cores of radially extending retraction fibrils.

Further degradation of a nerve cell is shown in fig 5. The cytoplasm is difficult to differentiate from the condensed nucleus. The cell periphery is profiled by small blebs, and the fibrous extensions of the cellular margin have apparently been degraded.

Discussion

A number of reports have shown that cultured nerve cells accurately reflect in-vivo phenomenon.[5-8] These cultured cells elaborate neuritic processes; they respond to voltage stimulation with typical action potentials similar to those generated in vivo; they form functional contacts with one another, and action potentials are transmitted to neighbouring neuronal cells. In addition, different types of nerve tissues that vary in structure and function show the same variations after in-vitro cultivation. Thus, the observations in this paper should be representative of in-vivo phenomenon.

The accompanying study[2] showed that *T pallidum* attached to cultured cells derived from dorsal root ganglia, superior cervical ganglia, and spinal cord. This study extends these findings to longer periods of incubation and demonstrates treponemal dysfunction and morphological damage to neuronal cells of dorsal root ganglia. This detrimental effect was dependent on attachment of treponemes. Heat-inactivated organisms failed to attach, and correspondingly nerve cell function and morphology were

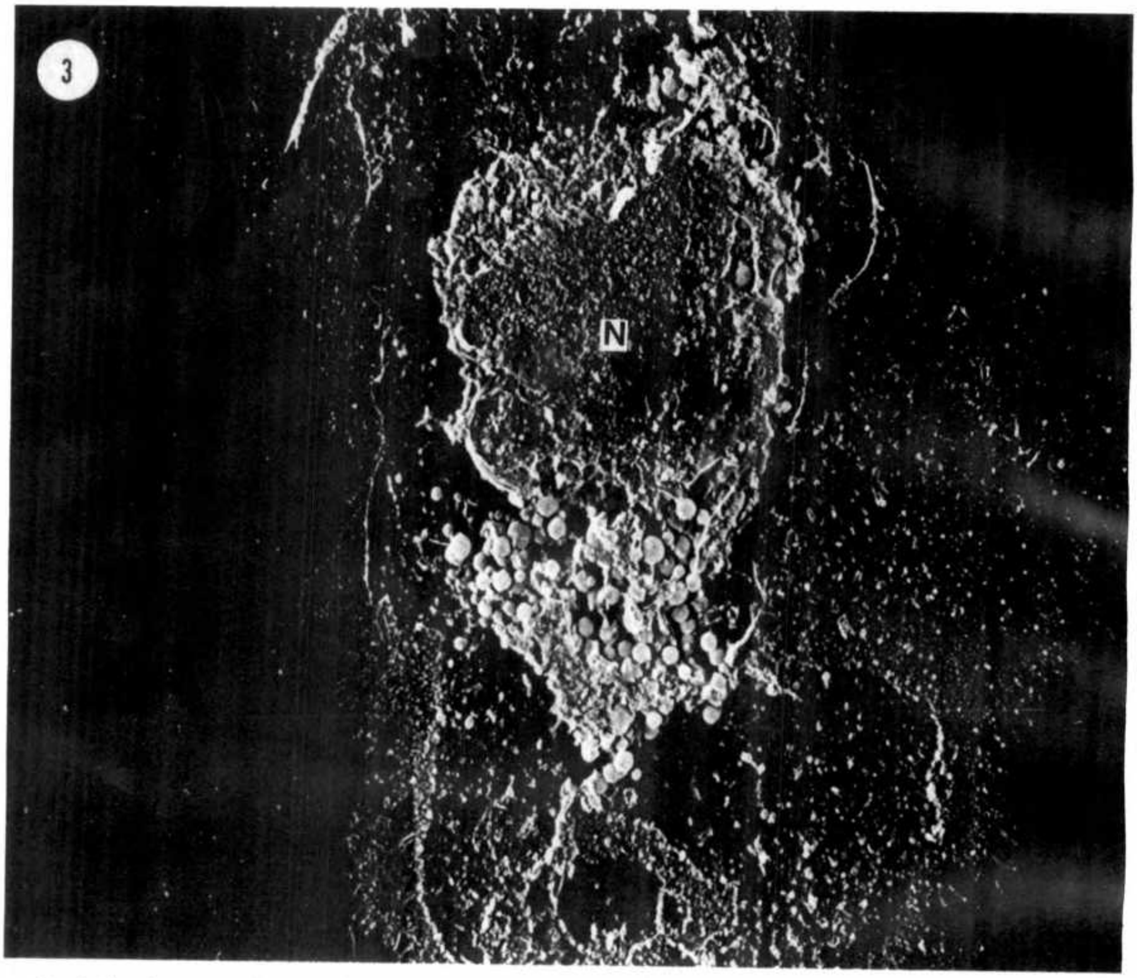

FIG 3 *A dorsal root ganglion cell degraded by extended incubation with T pallidum. The nuclear (N) profile is readily apparent. The cytoplasmic remnants are represented by an irregular fibrous meshwork and large spheres of various sizes. (× 1300).*

unchanged. Concentrations below 7×10^7 organisms/ml did not adversely influence nerve cell function or morphology. Damage at this, or lower concentrations, may have resulted if treponemal survival was extended. Without reducing agents, however, organisms retained motility in our system for about 48 hours. Most importantly, the concentrations that were detrimental (2×10^8 treponemes/ml) are consistent with syphilitic infection. The critical number of organisms required for the initial manifestations of erythema and induration is approximately 10^7 treponemes.[9] Assuming a generation time of 30 hours, within 4-5 days and before ulceration occurs, over 10^8 treponemes should be present. Neuronal cells lost their ability to respond electrophysiologically (8-10 hours) before morphological changes became apparent (16 hours). This is the first report of in-vitro nerve dysfunction and disruption attributed to the attachment of bacteria. A normal action potential requires an intact neuronal membrane. Thus, the first detectable damage must have reflected alterations in nerve cell membranes. With further incubation morphological damage occurred as manifested by holes in the nerve cell membrane and a flattening of the typical raised morphology. Further disruption of nerve cell integrity was characterised by condensation of the nucleus and an outlining of the cellular boundaries by fibrous cytoskeletal remnants. Similar morphological alterations have been shown in cells treated with Triton-X, a detergent that solubilises the plasma membrane,[10 11] or EGTA, a Ca^{++} specific chelator.

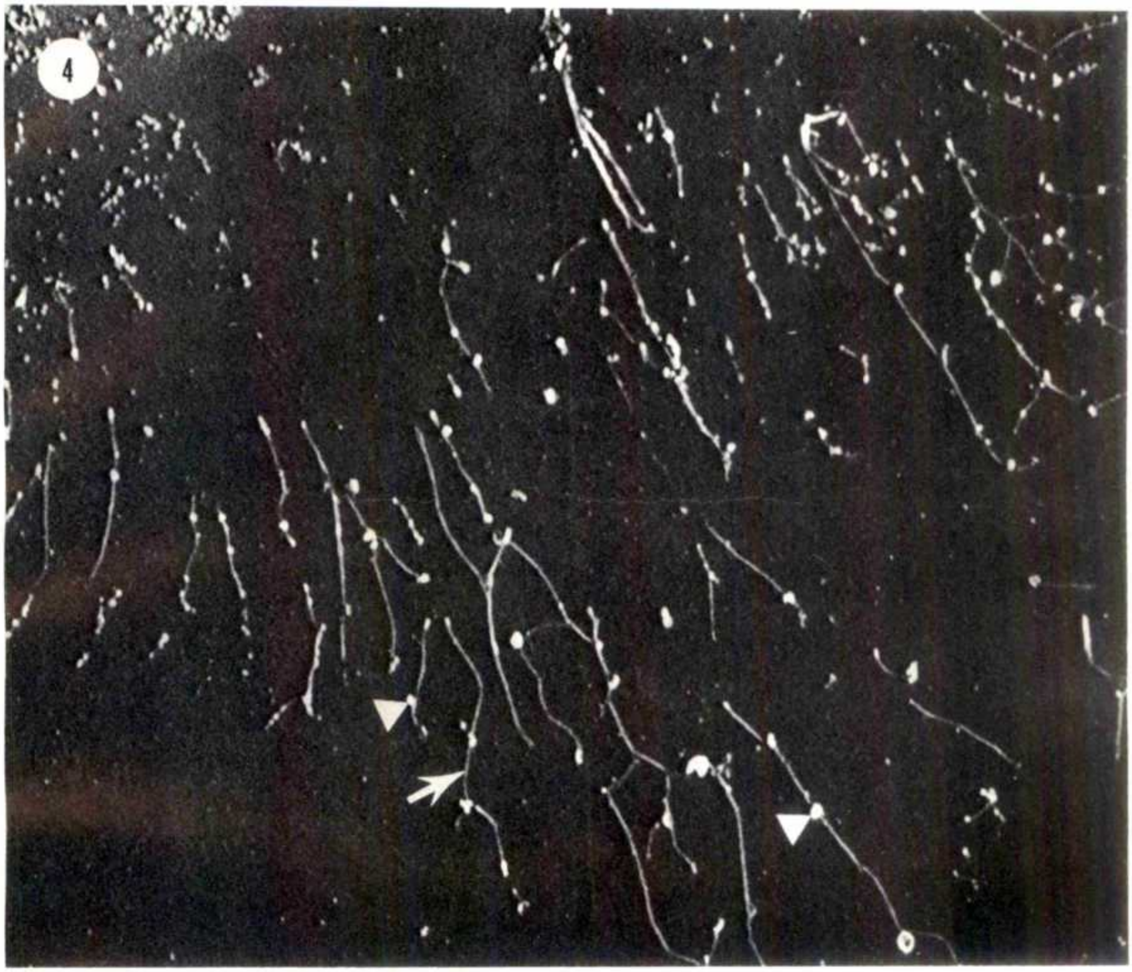

FIG 4 *A higher magnification of the cellular periphery of the nerve cell in fig 3. Note the cytoskeletal cores of retraction fibres (arrow) associated with bleb-like particles (arrowheads). (× 3400).*

These processes can be easily broken by gentle manipulation and separated from material that remains attached to the substratum. This substrate-attached material mediates cell adhesion and is primarily composed of fibronectin, glycosaminoglycans, and cytoskeletal proteins.[12] These areas of the cultured cells are apparently somewhat more resistant to degradation by *T pallidum*.

Oakes *et al*[13] recently reported similar detrimental influences of herpes viruses on dorsal root ganglia in culture. After viral inoculation alterations in electrophysiological indices were observed within four hours. Morphological alterations were detected after 16 hours; at this time less than half the neuronal cells responded with action potentials. Nerve cells were totally destroyed after 48 hours' incubation. The authors suggested that herpes virus reduced the sodium ion conductance of neuronal cells.

The findings in this study may partially explain two unknown factors of syphilitic infection: (a) the painless nature of most of the clinical manifestations of each stage of infection and (b) the severe nerve degeneration in tertiary and congenital syphilis. Two preliminary reports have shown that treponemes are associated with nerve fibres within lesion material.[1 14] In our tissue culture system after treponemal attachment to neuronal cells the initial effect was a functional impairment of the ability of these cells to generate an action potential. With further incubation morphological disruption of the neuronal cells was observed. Clinical manifestations of primary and secondary infection are usually evident for 2-6 weeks,

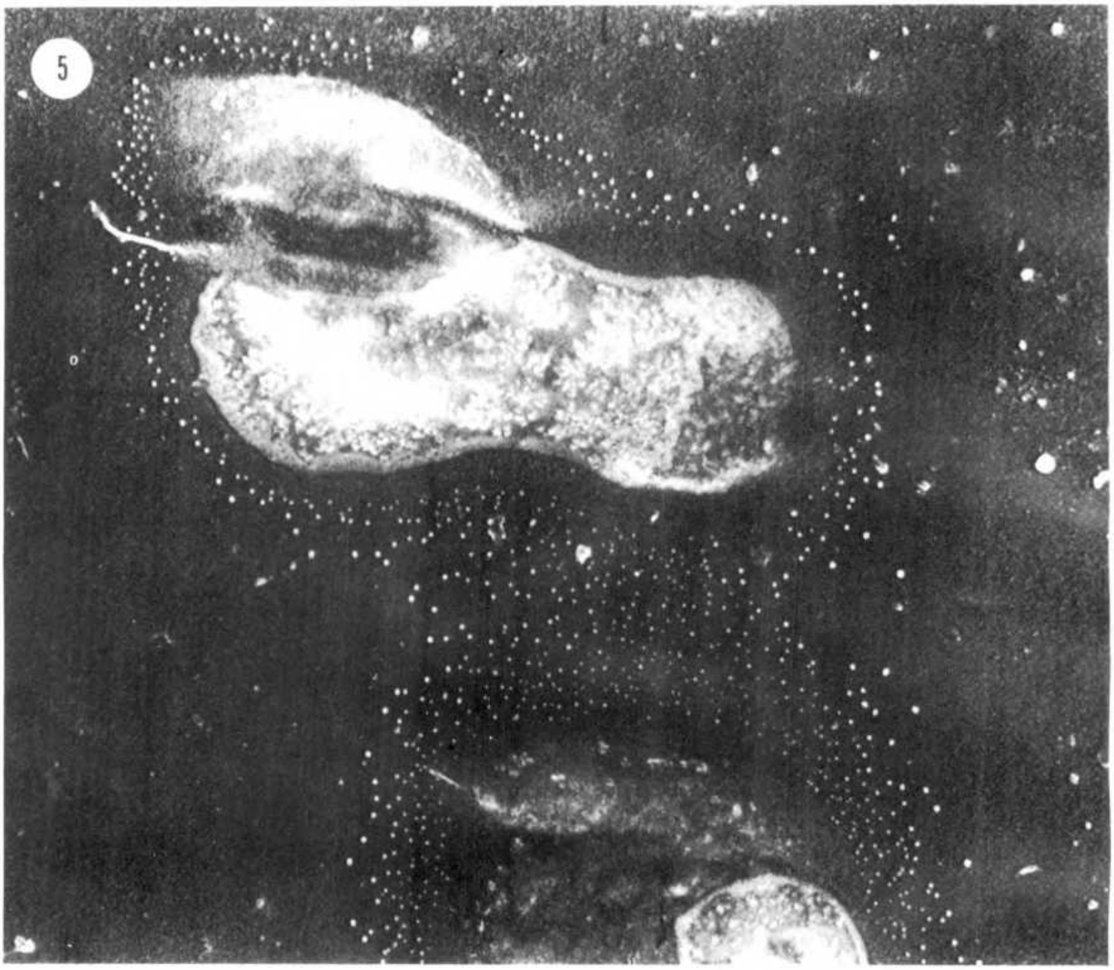

FIG 5 *Nerve cell incubated for 16 hours with T pallidum showing greatly altered morphology. The nuclear and cytoplasmic constituents are difficult to distinguish. The outline of the cell is marked by small cytoplasmic particles, probably remnants of attachment contacts. (× 1400).*

whereas manifestations of tertiary syphilis may last for years. In primary and secondary stages nerve deficits do not occur. The presence of treponemes within tissues for shorter periods of time (weeks) may interfere transiently with nerve function and account for the lack of pain. After healing pain receptors would return to normal without nerve deficits. In tertiary and congenital syphilis nerve damage frequently occurs. The presence of treponemes within tissues for longer periods (years) may result in morphological destruction causing damage to the central nervous system, particularly the cerebral cortex, spinal cord, and peripheral nerves.

We wish to acknowledge the excellent technical assistance of Paul Lima for tissue culture preparations, Eileen Gannon for treponemal preparations, and Anne Utyro for developing and processing the photomicrographs. This research was supported by the Public Health Service and National Institute of Allergy and Infectious Diseases grant AI 16-585, the National Institute of Neurological and Communicative Disorders and Stroke grant NS-1-325, and the Minnesota Medical Foundation grant 0762-5836.

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Br J Vener Dis 1982;**58**:228-30

Comparative in-vitro activity of selected new β-lactam antimicrobials against Neisseria gonorrhoeae*

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SUMMARY Four new β-lactam antimicrobials, ceftriaxone, cefotiam, cefonicid, and mecillinam, were evaluated in vitro against 72 β-lactamase-negative and 26 β-lactamase-positive isolates of *Neisseria gonorrhoeae*. Ceftriaxone was the most active of the antimicrobials tested. It inhibited all isolates, regardless of β-lactamase activity, at a concentration of $\leqslant 0{\cdot}015$ μg/ml. Cefotiam and cefonicid were also active against both groups but not as active as ceftriaxone. Both groups of *N gonorrhoeae* showed a high degree of resistance against mecillinam.

Introduction

The worldwide emergence of β-lactamase-positive strains of *Neisseria gonorrhoeae*[1] and the occasional occurrence of spectinomycin-resistant *N gonorrhoeae* strains[2] have prompted the search for new antimicrobial agents that might prove effective in treating infections caused by these resistant strains. Among the presently available newer β-lactam antimicrobial agents cefoxitin, cefuroxime, and cefotaxime have excellent in-vitro activity against *N gonorrhoeae*, including β-lactamase-positive strains.[3-5] Recent clinical studies concerning the use of these antimicrobial agents in treating gonococcal infections have shown encouraging results.[6-8] This study was conducted to evaluate the in-vitro activity of four new β-lactam antimicrobials, ceftriaxone, cefotiam, cefonicid, and mecillinam, against β-lactamase-negative and β-lactamase-positive strains of *N gonorrhoeae*.

Materials and methods

BACTERIAL STRAINS

A total of 98 strains of *N gonorrhoeae* were tested. Seventy-two β-lactamase-negative strains were collected during 1980 from patients with anogenital infections at the Hennepin County Medical Center, Minneapolis, Minnesota. Twenty-six β-lactamase-positive strains were obtained from the following sources: W Harrison, Naval Regional Medical Center, San Diego, California; W Hall, Veterans Administration Medical Center, Minneapolis, Minnesota; and the Center for Disease Control, Atlanta, Georgia. The identity of the isolates was confirmed by growth on Thayer-Martin agar, Gram stain, positive oxidase reaction, and acidification of glucose but not of maltose, lactose, or sucrose. The organisms were frozen in tryptic soy broth (Difco Laboratories, Detroit, Michigan) containing 20% glycerol and stored at −80°C. *N gonorrhoeae* isolates were tested for β-lactamase activity by an acidometric method.[9]

ANTIMICROBIALS

Ceftriaxone (RO-139904) and mecillinam (RO-109070) were obtained from Hoffman-La Roche Inc, Nutley, New Jersey; cefotiam (CGP14221/E) from CIBA-Geigy Corporation, Summit, New Jersey; and cefonicid (SK&F 75073) from Smith Kline and French Laboratories, Philadelphia, Pennsylvania. The antimicrobials were supplied as dry powders and stored at −20°C.

SUSCEPTIBILITY TESTING

The minimum inhibitory concentrations (MICs) of ceftriaxone, cefotiam, cefonicid, and mecillinam were determined by the agar dilution technique.[10] Two-fold dilutions of the antimicrobial agents, from

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*Paper read at the First Sexually Transmitted Diseases World Congress, San Juan, Puerto Rico, November 1981

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Accepted for publication 25 January 1982

8 to 0·015 μg/ml, were distributed into Mueller-Hinton agar supplemented with 2% haemoglobin and 1% IsoVitalex. The frozen gonococcal isolates were thawed and grown overnight on chocolate agar and then suspended in tryptic soy broth until the turbidity matched that of a 0·5 McFarland standard. One microlitre of a 1/10 dilution of the adjusted suspension (10^4 colony-forming units) was inoculated on to the plates containing the antimicrobial agent with a Steers replicator. The plates were incubated for 18 to 24 hours at 35°C in a CO_2 atmosphere. The minimum inhibitory concentration was defined as the lowest concentration of the antimicrobial agent that inhibited visible growth on the surface of the agar.

Results

The MICs of four antimicrobial agents against the β-lactamase-negative strains of *N gonorrhoeae* are given in table I. Ceftriaxone inhibited all isolates at a concentration of ⩽0·015 μg/ml. Both cefotiam and cefonicid were less active than ceftriaxone. MICs for 90% of the isolates of cefotiam were four-fold and of cefonicid eight-fold higher than those of ceftriaxone. These isolates showed a high degree of resistance to mecillinam.

TABLE I *Antimicrobial susceptibility of 72 β-lactamase-negative strains of N gonorrhoeae*

	MIC (μg/ml)		
		For strains (%)	
Antimicrobial agent	*Range*	*50*	*90*
Ceftriaxone	⩽0·015	⩽0·015	⩽0·015
Cefotiam	⩽0·015-1·0	⩽0·015	0·06
Cefonicid	⩽0·015-1·0	0·06	0·125
Mecillinam	0·125-8·0	2·0	4·0

The MICs of the four antimicrobial agents against β-lactamase-positive strains of *N gonorrhoeae* are shown in table II. Ceftriaxone was also highly effective against this group. All strains were inhibited at a concentration of ⩽0·015 μg/ml. Cefotiam and cefonicid were less active than ceftriaxone against these isolates. MICs for 90% of the isolates of cefotiam were eight-fold and of cefonicid 32-fold higher than those of ceftriaxone. This group was also highly resistant to mecillinam.

Discussion

Of the four new β-lactam antimicrobial agents tested in this study, ceftriaxone was found to be the most active against *N gonorrhoeae* regardless of β-lactamase production. These results agree with the study of Yoshikawa *et al*,[11] in which ceftriaxone was compared with cefuroxime, cefoxitin, and ampicillin against 87 β-lactamase-negative and eight β-lactamase-positive strains of *N gonorrhoeae*. Ceftriaxone was the most effective of the four antimicrobial agents tested. Cefonicid and cefotiam have also been tested previously against *N gonorrhoeae*.[12 13] The results were similar to those obtained in the present study. These two antimicrobial agents are quite active against *N gonorrhoeae;* however, they are not as active as ceftriaxone. Both cefonicid and cefotiam are presently undergoing clinical evaluation. Their role in the treatment of gonococcal infections remains to be determined. Mecillinam does not appear to be a potentially useful agent for the treatment of gonococcal infections. Although it is a β-lactam antimicrobial like the other three, it shows very poor activity against *N gonorrhoeae*. To our knowledge, the in-vitro activity of mecillinam against *N gonorrhoeae* has not previously been reported.

TABLE II *Antimicrobial susceptibility of 26 β-lactamase-positive strains of N gonorrhoeae*

	MIC (μg/ml)		
		For strains (%)	
Antimicrobial agent	*Range*	*50*	*90*
Ceftriaxone	⩽0·015	⩽0·015	⩽0·015
Cefotiam	⩽0·015-0·125	0·03	0·125
Cefonicid	0·06-0·50	0·25	0·50
Mecillinam	2·0-⩾8·0	⩽8·0	⩾8·0

In the present study MICs of ceftriaxone against *N gonorrhoeae* were similar to those for cefotaxime.[4 5] Cefotaxime has been shown to be highly effective against gonococcal infections due to β-lactamase-negative and β-lactamase-positive strains of *N gonorrhoeae*.[8] Ceftriaxone is presently undergoing clinical trials in the treatment of gonococcal infections. In a preliminary study uncomplicated gonorrhoea in men due to β-lactamase-negative strains was successfully treated with ceftriaxone in single intramuscular doses of 125 mg (15 patients), 250 mg (16 patients), and 500 mg (15 patients).[14] Among the new β-lactam antimicrobials in use or under clinical investigation for gonococcal infections, ceftriaxone appears to be the most promising because of its effectiveness at low doses. Further clinical evaluation of this antimicrobial for single-dose treatment of infections due to both β-lactamase-negative and β-lactamase-positive strains of *N gonorrhoeae* is warranted.

Sue Counter assisted in the preparation of the manuscript.

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Br J Vener Dis 1982;**58**:231-5

Treatment of uncomplicated gonorrhoea in women

Comparison of rosoxacin and spectinomycin

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SUMMARY A comparative study of the new antibacterial agent, rosoxacin, a quinoline derivative, with spectinomycin was made in women with uncomplicated cervical, urethral, pharyngeal, and rectal gonorrhoea. Rosoxacin was given in three oral regimens: 200 mg in a single dose, 300 mg in a single dose, and 300 mg in two doses of 150 mg four hours apart. All culture results 72 hours after administration were negative for *Neisseria gonorrhoeae* in all 81 women compared with 107 of 109 who received 2 g spectinomycin intramuscularly.

Thirty-five of the women successfully treated with rosoxacin harboured penicillinase-producing strains of *N gonorrhoeae* (PPNG) and 46 non-penicillinase-producing (non-PPNG) strains. Fifty of the women treated with spectinomycin had PPNG strains and 59 non-PPNG strains. Mild self-limiting side effects, principally dizziness, occurred in varying frequency with rosoxacin, but these were difficult to evaluate owing to the characteristics of the patient population and the conditions under which the study was conducted.

Introduction

The emergence in 1957 of gonococcal strains with decreased sensitivity to penicillin[1,2] and in 1979 of penicillinase-producing *Neisseria gonorrhoeae* (PPNG)[3,4] together with recent reports of spectinomycin-resistant mutants,[5,6] particularly in PPNG,[7] have created an urgent need for alternative treatment regimens for gonococcal infections. Rosoxacin, 1-ethyl-1, 4-dihydro-4-oxo-7 (4-pyridyl)-3 quinoline carboxylic acid, is a new synthetic agent with good in-vitro activity against *N gonorrhoeae*, both PPNG and non-penicillinase-producing (non-PPNG) strains. Clinical data show that its use in gonococcal infections is promising but they need to be expanded.[8-10] This study was thus designed to evaluate the clinical efficacy and tolerance of various doses of rosoxacin and to compare them with those of spectinomycin in the treatment of uncomplicated gonorrhoea in women.

Patients and methods

STUDY POPULATION

The study population consisted of prostitutes with uncomplicated gonococcal infection seen at the Balibago Social Hygiene Clinic, Angeles City, between June and August 1980. Patients were enrolled in the study if urethral or cervical screening cultures grew *N gonorrhoeae*. Written informed consent was obtained from each patient before random allocation to a treatment group using a table of random numbers.

Criteria for exclusion from the study included azoquinoline allergy, serious systemic disease, or antimicrobial treatment within the previous week. Women who were pregnant were included only in the group treated with spectinomycin.

STUDY DESIGN

Dose-ranging study

Patients were allocated to treatment with rosoxacin or spectinomycin according to a table of random numbers. A total of 81 women received oral rosoxacin; 32 were given a 200-mg single dose, 21 a 300-mg single dose, and 28 300 mg in two doses of 150 mg four hours apart. One hundred and nine

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Accepted for publication 15 February 1982

P27654

women received 2 g spectinomycin intramuscularly. The rosoxacin and spectinomycin groups were comparable in age (18-25 years) and weight (38·5-73·0 kg).

Rosoxacin tolerance study

A double-blind crossover oral tolerance study was undertaken in 30 patients divided into two groups of 15 women each. One group (group A) received 300 mg rosoxacin as a single dose on day 1 and a matching placebo tablet on day 2 whereas the other group (group B) received placebo on day 1 and a single dose of 300 mg rosoxacin on day 2.

TREATMENT RESPONSE AND SIDE EFFECTS

The patients were treated in a large ward during the study to ensure follow-up and preclude reinfection before evaluation of treatment results. All patients were asked about their alcohol intake and drug habits, but accurate histories were not always obtainable because of the unreliability of the patient population and their reluctance to admit to the use of drugs.

Cases included in the final analysis of efficacy consisted only of those who had positive pretreatment culture results and interpretable post-treatment culture results. Treatment was considered a failure if *N gonorrhoeae* was isolated from any of the post-treatment cultures.

During the three days of treatment, the patients were interviewed and examined at frequent intervals for signs and symptoms of possible side effects. Direct questions about specific symptoms, particularly those related to the central nervous system, were asked and a positive or negative response obtained. All patients were questioned in the presence of others. At no time in the study was there a need to break the code because of side effects or for other reasons. Side effects were monitored in all patients studied.

LABORATORY TECHNIQUES

Before treatment and three days after, culture specimens were obtained with a cotton swab from the pharynx, urethra, endocervix, and rectum. Specimens were inoculated directly on to modified Thayer-Martin medium and incubated at 35°C in an atmosphere of 5% carbon dioxide. Gonococci were identified by colonial morphology, Gram stain, and oxidase reaction. Isolates from the pharynx were confirmed by sugar fermentation tests. All strains were tested for β-lactamase production by the chromogenic cephalosporin test.[11] Minimum inhibitory concentrations (MICs) of rosoxacin were determined by the standard dilution technique for 88 isolates.[12]

Laboratory monitoring for rosoxacin toxicity included complete blood counts, urine analysis, and measurements of blood urea nitrogen, serum creatinine, serum aspartate aminotransferase, and glucose 6-phosphate dehydrogenase before and 24 hours after treatment. Electrocardiograms were obtained in 20 patients who received a single dose of 300 mg rosoxacin at six and 24 hours after treatment.

Results

SENSITIVITY OF GONOCOCCAL ISOLATES TO ROSOXACIN

The cumulative susceptibility to rosoxacin of 30 PPNG and 58 non-PPNG strains is shown in table I. The range of MICs for the isolates was 0·000001-0·06 μg/ml, with a mean of 0·015 μg/ml for PPNG strains and 0·03 μg/ml for non-PPNG strains. Only one PPNG isolate had an MIC of 0·06 μg/ml of rosoxacin.

TABLE I *Cumulative percentage of N gonorrhoeae isolates inhibited by varying concentrations of rosoxacin*

MIC (μg/ml)	*PPNG*	*Non-PPNG*
0·000001	3	3
0·000002	7	3
0·000005	7	5
0·00001	7	9
0·00002	17	19
0·00005	23	21
0·0001	27	24
0·0002	27	33
0·0005	33	34
0·001	43	50
0·002	50	55
0·005	60	62
0·008	73	74
0·01	83	83
0·03	100	98
0·06		100

MIC = minimum inhibitory concentration; PPNG = pencillinase-producing *N gonorrhoeae;* non-PPNG = non-penicillinase-producing *N gonorrhoeae*

TREATMENT

Rosoxacin

Cervical, urethral, rectal, and pharyngeal culture results after treatment were negative in all 81 patients treated with the three regimens of rosoxacin (table II). All had urethral and endocervical infections; additionally, two patients had a pharyngeal infection while 34 had a rectal infection. Thirty-five (43%) patients with infection due to PPNG strains were cured; 17 received a 200-mg single dose, seven a 300-mg single dose, and 11 a 300-mg split dose. Similarly, all 46 (57%) patients with non-PPNG strains were cured; 15 received a 200-mg single dose, 14 a 300-mg single dose, and 17 a 300-mg split dose.

TABLE II *Efficacy of rosoxacin and spectinomycin treatment in uncomplicated gonococcal infections*

Regimen	*PPNG* *No treated*	*% Cured*	*Non-PPNG* *No treated*	*% Cured*
Rosoxacin				
200-mg single dose	17	100	15	100
300-mg single dose	7	100	14	100
300-mg split dose	11	100	17	100
Spectinomycin	50	100	59	97

PPNG = penicillinase-producing *N gonorroeae;* non-PPNG = non-penicillinase-producing *N gonorrhoeae*

Spectinomycin

Cervical, urethral, rectal, and pharyngeal culture results after treatment were negative in all but two of the 109 patients treated with spectinomycin (table II). Of those who responded to treatment, all had urethral and endocervical infections; additionally, one had a pharyngeal infection and 32 rectal infections. The difference between the cure rates with rosoxacin and spectinomycin was not statistically significant ($\chi^2 = 0\cdot29$; $P>0\cdot05$). There was no difference in the symptomatic response of the two groups. Of those with evaluable symptoms, two of the 51 (4%) patients treated with spectinomycin showed no improvement in symptoms compared with two of the 50 (40%) patients treated with rosoxacin. In both groups those who did not respond symptomatically nonetheless had negative post-treatment culture results for *N gonorrhoeae.*

SIDE EFFECTS

Rosoxacin

The incidence of apparent side effects reported by patients receiving one of the three dosages of rosoxacin are given in table III. Eleven patients were excluded from the evaluation of side effects, since 10 were already taking drugs which affected the central nervous system. Nineteen of 25 (76%) patients taking a single dose of 300 mg, 20 of 30 (67%) taking a split dose of 300 mg, and 17 of 49 (35%) taking a single dose of 200 mg reported one or more side effects. The differences in side effects with the three regimens were statistically significant ($\chi^2 = 14\cdot17$, $P<0\cdot05$) because of a significant difference in the side effects noted with the 200-mg single dose compared with the 300-mg single dose ($\chi^2 = 11\cdot31$, $P<0\cdot05$) and 300-mg split dose ($\chi^2 = 7\cdot64$, $P<0\cdot05$); there was no difference in the incidence of side effects between the 300-mg single dose compared with the 300-mg split dose ($\chi^2 = 0\cdot56$, $P>0\cdot05$).

In all but three instances, these side effects were mild and self-limiting. In all but one of the patients they affected the central nervous system: dizziness in 53, vertigo in 20, drowsiness in 17, headache in 19, euphoria in two, and hallucination in one. In addition, two patients taking a single dose of 300 mg had blurred vision. Nausea was reported by five patients taking a single dose of 300 mg, three a split dose of 300 mg, and two a single dose of 200 mg. Most patients with dizziness had mild symptoms and only those taking a single dose of 300 mg (three patients) reported "severe" dizziness. Apart from this there was no correlation between the intensity of the dizziness and the dose of rosoxacin. In most patients the duration of dizziness was less than four hours with all regimens. The incidence of more prolonged dizziness (over four hours) was not correlated with dosage. The symptoms were generally well tolerated and no treatment or restriction of activity was required. No changes in vital signs or physical findings occurred that were thought to be related to the drug.

TABLE III *Side effects in 104 patients taking three different doses of rosoxacin*

	Rosoxacin *200-mg single dose*	*300-mg single dose*	*300-mg split dose*	*Total**
No enrolled	50	26	39	115
No evaluated	49	25	30	104
No (%) with one or more adverse reaction	17 (35)	19 (76)	20 (67)	
No (%) with adverse reaction of the CNS	17 (35)	18 (72)	20 (67)	
No (%) with blurred vision	0 (0)	2 (4·1)	0 (0)	
No (%) with nausea	2 (4.1)	5 (20)	3 (10)	

CNS = central nervous system

*The total of 115 enrolled includes 81 patients who were evaluated for efficacy as well as 34 who did not meet the criteria for efficacy but were evaluated for side effects

Spectinomycin

Side effects after spectinomycin treatment occurred in 19 of 187 (10%) patients evaluated for safety. These included moderate pain at injection site in nine, headache in eight, dizziness in five, and rashes with pruritus in one.

ROSOXACIN TOLERANCE STUDY

Of the 15 patients in group A who received 300 mg rosoxacin on day 1, four developed side effects within 24 hours after rosoxacin treatment while one had side effects on day 2 after receiving the placebo. Of the 15 patients in group B who received a placebo on day 1, one developed symptoms on day 1 within 24 hours of administration, while seven developed symptoms on day 2 within 24 hours of receiving rosoxacin. Thus, only 11 (37%) of those included in the double-blind tolerance study developed side effects after treatment with rosoxacin. All the 30 patients studied had negative culture results for *N gonorrhoeae* after treatment.

LABORATORY TESTS

No significant changes were noted in blood counts, blood urea nitrogen, serum creatinine, or glucose 6-phosphate dehydrogenase after treatment with rosoxacin. A slight transient increase in serum aspartate transaminase values occurred in six patients. Mild transient haematuria was noted in four patients and pyuria increased in eight. Of the 20 patients given a 300-mg single dose in whom electrocardiography was performed, two developed sinus arrhythmia six hours after treatment; in one patient this arrhythmia had reverted to normal 18 hours later.

Discussion

All 81 cases of uncomplicated gonorrhoea in these female patients, including 35 PPNG infections, were cured by rosoxacin. This shows that all the three regimens were effective in producing a bacteriological cure. Our data conform with those of earlier studies showing a 100% cure rate with a 300-mg dose. The result with the 200-mg dose was better than the 66·7% cure rate previously reported[8] and significantly better than the results obtained in male patients where no cure was noted in eight cases (unpublished data). A lower dose of 100 mg has been tried in Europe, but in the first three cases treatment failed; thus it is considered to be unacceptable.[8] The clinical response to rosoxacin was consistent with its excellent in-vitro activity, with MICs of 0·06 μg/ml or less for non-PPNG as well as for PPNG strains.

This study was undertaken in a locality where PPNG accounts for 30-40% of gonococcal infections,[13-15] which could not have been eradicated by penicillin treatment. This high incidence has led to the increased use of spectinomycin, which may result in the selection of spectinomycin-resistant mutants.[7 16 17] Our findings showed that rosoxacin, either in a 200-mg single dose, 300-mg single dose, or 300-mg split dose, compared favourably with spectinomycin in the treatment of uncomplicated gonorrhoea in women. Since rosoxacin may be given orally in a single dose it appears to be a reasonable alternative to spectinomycin, particularly when penicillin-resistant gonorrhoea is prevalent.

An obvious disadvantage of this drug is the high incidence of side effects, as seen in this study. The apparent incidence appeared to increase with dosage. The frequency of apparent side effects in the double-blind tolerance study, however, was about one-half that in the open-dose study among patients who received the same 300-mg single dose of rosoxacin. The discrepancy between these two groups, studied under the same conditions and taking the same dosage, cannot be explained, and may well illustrate the difficulty in drawing conclusions from these findings. Moreover, the incidence of reported side effects in this study was much higher than that found in other studies conducted in several hundred patients in Europe, the USA, South America, and other Asian countries (unpublished data). In addition, a lower incidence of side effects of 14% was noted in Filipino men receiving a 300-mg single dose in a concurrent study.[18] Although the difference in mean body weights between the male and female Filipinos (65·6 kg compared with 47·5 kg respectively) might be considered to be a contributory factor, there appeared to be no correlation between apparent side effects and body weight within the group of women receiving a single dose of 300 mg rosoxacin. The mean body weight in those reporting notable side effects was 46·7 kg compared with 46·2 kg in those with no or negligible apparent side effects. Thus, the influence of body weight is not clear.

The evaluation of these apparent side effects, primarily dizziness, was difficult because of the very nature of the study population and the methods used to determine them. These limitations were to be expected since the study was undertaken among prostitutes, who are highly susceptible to suggestion and yet in whom only direct questioning could be used to obtain answers about side effects. The fact that alcohol and drug abuse are indispensible features of their profession prevent any definitive conclusions about the frequency, type, and intensity of side effects attributable to rosoxacin in this study, since by themselves these two abuses may account for some of the side effects complained of. What is clear is that the side effects are generally mild and self-limiting.

This project was supported by the National Research Council of the Philippines and the Winthrop-Stearns Philippines. The study was conducted in accordance with the guidelines for human experimentation set forth by the Declaration of Helsinki and the Committee on Research, Ethics and Development of the UP Health Sciences Center.

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Br J Vener Dis 1982;**58**:236-8

Provision of a chlamydial culture service to a sexually transmitted diseases clinic

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SUMMARY Urethral specimens from 215 men were inoculated on to McCoy cell cultures, both at the local laboratory and at a central reference laboratory. *Chlamydia trachomatis* was isolated from 58 (28%) patients; 12 of these isolates were, however, obtained only at the local laboratory. The results show the feasibility and convenience of a central laboratory supplying a peripheral laboratory with uninoculated prepared cell cultures. Such a service is not only more cost effective but obviates the problems of transporting specimens to a central laboratory.

Introduction

Chlamydia trachomatis is now recognised as an important pathogen affecting the genital tract of men and women.[1 2] Owing to cost and the need for a tissue culture laboratory, however, a routine culture service for this organism has not been widely available outside research centres. The arguments for and against the free availability of such a service have been discussed in detail.[3-6] All workers agree that, at the very least, a partial service for selected groups of patients should be more widely available.

Virus diagnostic laboratories tend to be localised on a regional basis, and it is in these departments that tissue culture expertise is readily available. We, therefore, examined the feasibility of a central reference laboratory sending prepared coverslip cell cultures to a peripheral diagnostic laboratory for inoculation, incubation, and interpretation.

Patients and methods

Unselected male patients attending a sexually transmitted diseases (STD) clinic (Royal Northern Hospital) with urethral discharge were included in the study. Patients receiving antimicrobial chemotherapy within the previous four weeks were excluded. Specimens were taken from the distal urethra for Gram stain and culture for *Neisseria gonorrhoeae*. A cottonwool-tipped wire swab was inserted 4 cm into the urethra, rotated, and removed. The tip was cut off into chlamydia transport medium[7] screened with gentamicin, vancomycin, and amphotericin B. Inoculated specimens were held at 4°C until transported to the local laboratory in the same hospital (usually within the working day). On arrival, one half of the transport medium was decanted and sent to the central laboratory (University College Hospital), usually within 24 hours. Both specimens were inoculated on to IUDR-treated McCoy cells prepared at the central laboratory by the method described.[7] Prepared coverslip monolayers were sent to the local laboratory from the central laboratory at twice weekly intervals. After centrifugation and incubation coverslip preparations were stained with Lugol's iodine and examined by light microscopy. Neither laboratory was at this stage aware of the other's findings. All slides were subsequently reviewed at the central laboratory and any discrepancies checked by a third observer. Statistical correlates were calculated using the χ^2 test with Yates's correction.

Results

Specimens were obtained from the urethras of 215 men. Seven of these specimens disrupted both laboratories' cell sheet. A further two specimens caused disruption of the local laboratory cell culture

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Accepted for publication 8 February 1982

TABLE I *Isolation of C trachomatis from urethral specimens with and without delayed inoculation*

Culture and interpretation by:				
Local laboratory	*Central laboratory (of same sample)*	*Central laboratory*	*Total (%)*	*% Of confirmed positives*
+	+	+	38 (18·45)	69
+	+	−	12 (5·83)	21·8
+	−	−	3 (1·46)	
−	+	−	2 (0·97)	3·63
−	−	+	3 (1·46)	5·45
−	−	−	148 (71·84)	
Total			206	

+ = Positive; − = negative

only. The central laboratory culture for these two latter specimens gave a negative result for *C trachomatis*. Thus, specimens from 206 men were available for comparison. Of these patients, 18 (8·7%) had gonococcal urethritis and 188 non-gonococcal urethritis.

C trachomatis was isolated from 58 (28·2%) of the specimens; in 38 the organism was isolated from cultures at both laboratories (table I). The local laboratory culture alone yielded *C trachomatis* from 12 specimens and the central laboratory culture alone yielded *C trachomatis* from three specimens. Three cultures reported as positive by the local laboratory were on review at the central laboratory thought not to grow *C trachomatis*, and two cultures reported as negative by the local laboratory were positive on review.

Failure to isolate *C trachomatis* at the central laboratory, although the local laboratory cultures gave positive results, was not related to the delay between collection of the specimen and the inoculation of the cell monolayer. There was, however, a strong association between the inclusion count in the local laboratory specimen and the isolation of *C trachomatis* from both specimens (table II). This relationship was particularly significant when inclusion counts of <25 were compared with those of >25 ($P < 0·001$).

TABLE II *Relationship of inclusion count to positivity in 48 specimens growing C trachomatis*

	C trachomatis isolated from:	
Inclusion count	*First specimen only*	*Both specimens*
>300	2	15
<300	14	17
Total	48	(0·05>P<0·02)
>25	5	27
<25	11	5
Total	48	(P<0·001)

Discussion

C trachomatis is an important cause of genital infection in both men and women, and its accurate diagnosis in STD clinics is highly desirable. Often specimens are sent to a central laboratory for processing. Our results, however, show that over 20% of the *C trachomatis* isolates would not have been detected if the specimens had been processed at the reference laboratory. Thus, apart from the convenience of culturing the specimen locally, the yield was considerably increased. The discrepancies between the two laboratories' interpretation of the same coverslip concerned low inclusion counts, which might have been subject to observer error under normal conditions. Of the three specimens giving positive results only in the central laboratory culture, two had low inclusion counts (<10/coverslip) and the remaining culture >300 inclusions/coverslip.

The disruption of the cell sheet in nine cultures did not appear to be due to excess growth of *C trachomatis*, as in two specimens the central laboratory culture result was negative without disruption of the cell sheet. This finding is in line with the toxin theory of Ngeow *et al.*[8]

The finding that the number of inclusion-forming units in the initial inoculum was related to the ability to isolate the organism after transport implied that the antigenic load was the limiting factor rather than delay in inoculating cell cultures. We have found this to be true of specimens from the eyes of babies with chlamydial ophthalmia (where the antigenic load is often very high), which are more likely to survive transport to a distant laboratory than specimens from the adult genital tract.

The bulk preparation of coverslip cultures is more economical of staff and materials than production by a number of smaller units. Culture preparation and the maintenance of cell lines are the most time consuming aspects of the isolation of *C trachomatis*, since they require a high level of ability in tissue culture techniques. In contrast, the inoculation, incubation, and interpretation of cultures are within the capabilities of the trained staff of a routine diagnostic laboratory and do not require expensive equipment. The provision of a temperature-controlled centrifuge is desirable, but such a piece of apparatus will have other uses within the department. A proper technique of specimen collection is important or a low (and potentially misleading) isolation rate will be obtained.

Large-scale screening methods for *C trachomatis*, such as described by Mallinson *et al*,[9] do not overcome the problems of transport and the need to store specimens before inoculation. The provision of prepared cell cultures from a central laboratory makes the culture of *C trachomatis* economic and convenient. Taking the cultures to the patient, as suggested by Ngeow *et al*,[8] might provide a useful refinement to this technique.

We thank the staff of the Microbiology Departments and Sexually Transmitted Diseases Clinics at University College Hospital and the Royal Northern Hospital for their assistance and co-operation with this study.

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Br J Vener Dis 1982;**58**:239-42

Characteristics of Haemophilus ducreyi

A study

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SUMMARY In a study of 13 local and four reference strains of *Haemophilus ducreyi* all grew well on a selective medium consisting of Bacto proteose No 3 agar (Difco), soluble starch, IsoVitalex, human blood, and vancomycin. All the strains reduced nitrate, were alkaline-phosphatase-positive, and (with one exception) used glucose, fructose, and mannose. β-lactamase was produced by 12 local strains. Erythromycin was the most effective antibiotic tested, followed by streptomycin, co-trimoxazole, and spectinomycin.

Introduction

Chancroid has been traditionally regarded as a tropical disease which rarely occurs in temperate countries. In recent years, however, there have been reports of its incidence in Greenland,[1] Canada,[2] the United States,[3 4] and Britain.[5] This is largely due to the introduction of a selective medium for the isolation of *Haemophilus ducreyi.*[6]

Despite the greater interest being shown in the disease, some of the characteristics of the organism are still not established. In this paper we report the characterisation of 13 local strains, which were isolated in 1979. A reference strain (CIP 54.2) from the Institut Pasteur, Paris, and three other strains (C1, C2, and C3) (obtained by courtesy of W L Albritton, Canada) were included in the study. Our findings suggest that *H ducreyi* is able to utilise carbohydrates and reduce nitrate. The oxidase reaction was weak, but this could have been due to the non-specific action of haemin.

Materials and methods

CULTURE MEDIA

The medium used to grow the organism was made up of Bacto Proteose No 3 agar (Difco), to which was added soluble starch 0·1%, IsoVitalex 1%, human blood 15%, and vancomycin 3 μg/ml. Plates of medium were sealed in a plastic bag and kept at 4°C until use within a week. Cottonwool swabs saturated with normal saline were used to collect culture samples from the penile ulcers of patients presenting with clinically diagnosed chancroid. Specimens were inoculated directly on to the plates of medium, which were then kept in a candle-extinction tin together with a piece of moist cottonwool. The plates were incubated at 35°C and read after 48 hours and daily thereafter. The strains were identified presumptively by their colonial morphology and by microscopy. The liquid medium which was used to grow the organism consisted of Bacto Proteose Peptone No 3 (Difco) 1%, sodium chloride 0·5% and fetal bovine serum 10%.

BIOCHEMICAL TESTS

Tests for oxidase,[7] alkaline phosphatase,[8] catalase, the ability to biosynthesise porphyrin from δ-aminolaevulinic acid (ALA),[9] and β-lactamase[10] have been described. To improve the oxidase reaction 24-hour cultures of the organisms were suspended in phosphate-buffered saline pH 7·2 and centrifuged.[11] The supernatant fluid was removed and the sediments deposited on a piece of filter paper. Oxidase reagent was then dropped on to the sediments and the changes observed. The activity of haemin on the oxidase reagent, NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride, was determined by preparing a 20% solution of haemin (Sigma H-2375 equine type III) in 1 mol/l sodium hydroxide and diluting it in distilled water to give a final concentration of 800 μg/ml. This dilution was dropped on to one end of a strip of filter paper, and to the other end was added a drop of oxidase reagent. The development of a purple colour was looked for at the interface between the two reagents.

The requirement for V factor was tested by the ability of the organisms to grow on a medium made up of GC medium base (Difco), haemoglobin 1%,

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Accepted for publication 17 January 1982

glucose 0·1%, and L-glutamine 0·01%. Nitrate reduction was tested by suspending a heavy inoculum of the organism in a liquid medium consisting of Bacto Proteose Peptone No 3 (Difco) 1%, sodium chloride 0·5%, soluble starch 0·1%, and potassium nitrate 0·1%. After six hours' incubation at 35°C, reagents A and B were added as described.[12]

Carbohydrate utilisation was tested by a reagent-impregnated filter paper method (unpublished data). Briefly, the reagents consisted of Bacto Proteose Peptone No 3 (Difco) 1%, L-cysteine 0·026%, carbohydrate 5%, and bromocresol purple 0·2% dissolved in 0·025 mol/l Sorensen's phosphate buffer pH 7·2. Pieces of Whatman's No 1 filter paper, measuring 4 × 1 cm were placed on Petri dishes and saturated with the respective reagents. Heavy inocula of 24-hour organisms were then smeared on the surface of the paper strips. Pieces of wet cottonwool were also deposited on the Petri dishes, which were then placed in a tin containing some water. The lid was closed and the tin incubated at 35°C for two hours. Carbohydrate utilisation was indicated by the presence of yellow zones around the inocula. The paper remained purple if there was no utilisation. The carbohydrates tested were arabinose, xylose, glucose, fructose, mannose, galactose, lactose, maltose, and sucrose.

Other biochemical reactions were also tested using the MICRO-ID (General Diagnostics) system for identifying members of the *Enterobacteriaceae*. The system tested for Voges-Proskauer reaction, nitrate reduction, phenylalanine deaminase, hydrogen sulphide, indole, ornithine decarboxylase, lysine decarboxylase, malonate utilisation, urease activity, esculin hydrolysis, β-galactosidase, and fermentation of arabinose, adonitol, inositol, and sorbitol. The manufacturer's directions were followed except in the inoculation of the organism. The clear plastic tape covering the test wells was cut away and the organism directly inoculated on to the test discs; 0·2 ml normal saline was then dropped on to each disc and a transparent sealing tape used to cover the test wells.

SENSITIVITY TESTS

The minimum inhibitory concentrations (MICs) of ampicillin, erythromycin, tetracycline, streptomycin, sulphadiazine, co-trimoxazole (trimethoprim: sulphamethoxazole ratio, 1 : 19), spectinomycin, vancomycin, colistin, and amphotericin B were determined by the agar dilution technique of Hammond *et al*[14] using GC medium base (Difco), haemoglobin 1%, IsoVitalex 1% (except for sulphadiazine and co-trimoxazole where Enrichment 4[15] and glucose 0·1% were used) and doubling dilutions of antimicrobial agents.

Results

Good growth of all the strains was obtained with the medium described. In the early phase of our study various types of media were used, some of which were too inhibitory. Different types of agar had varying effects on *H ducreyi*. Thus, better growth was obtained using Bacto purified (Difco) or Baltimore Biological Laboratory (BBL) purified agar than BBL granulated or Oxoid agar No 1. In the formulation chosen soluble starch was unnecessary for all the strains except CIP 54.2, which grew better with it.

COLONIAL GROWTH

Most of the strains produced colonies of 0·5-1 mm in diameter in 24 hours and of 1-2 mm in 48 hours. They were whitish in colour but might have a pale yellow-brown hue in the heaped up areas where the growth was confluent. They could be pushed intact across the agar surface. It was difficult to make a uniform suspension of the colonies in a drop of water as the organisms were stuck together in clumps. In broth culture granular growth was seen after 24-48 hours. The clumps tended to adhere to the side of the testtube. After 3-5 days long filaments were seen, giving a woolly appearance.

MICROSCOPICAL EXAMINATION

Microscopical examination of Gram-stained colonies from solid medium showed that few of the organisms occurred as isolated Gram-negative bacilli. Most of them were present as members of microcolonies. When 24-hour cultures were examined, the organisms at the edge of some of the microcolonies tended to be arranged in parallel rows, giving a wavy appearance. Long chains of organisms were more evident when the cultures were taken from liquid medium. These were sometimes arranged in loops, and occasionally parallel rows produced the classical "railway track" appearance.

BIOCHEMICAL REACTIONS

All the strains grew in the absence of V factor and were unable to biosynthesise porphyrin from ALA. They were all catalase-negative but positive for alkaline phosphatase and nitrate reduction. The oxidase reaction was slow to develop with the traditional method.[7] After 10-15 minutes a pale purple colour appeared on and around the inocula; the colour darkened with time. When the cells were first washed in phosphate-buffered saline, the purple colour developed within 3-4 minutes of the reagent being added. On the other hand, haemin itself was also able to turn the oxidase reagent purple within a few minutes. When a small piece of uninoculated medium was added to the filter paper, a similar darkening of the agar occurred on the undersurface.

Sixteen of the 17 strains were able to utilise glucose, fructose, and mannose by the filter paper method. The reference strain CIP 54.2 was unable to utilise any of the carbohydrates. For consistent results heavy inocula from 24-hour cultures were used. A high humidity was necessary to reduce evaporation to a minimum. There was no difficulty in distinguishing a positive yellow zone from a pallor around some of the inocula due to excess moisture. All the strains gave similar results for the MICRO-ID system. Of the 15 tests, only nitrate reduction gave a positive result.

β-lactamase was produced by 12 local strains and none of the reference strains (table). All the β-lactamase producers had MICs for ampicillin >8 μg/ml. Erythromycin was the most effective antibiotic, followed by streptomycin, co-trimoxazole, and spectinomycin. A high percentage of the strains was resistant to tetracycline, although all were uniformly resistant to vancomycin and amphotericin B.

Discussion

The isolation of 13 strains of *H ducreyi* from genital ulcers confirms the value of a selective solid medium for diagnosing chancroid. The organism is fastidious in its growth requirements. The optimum medium should,. therefore, be able to provide adequate hydration, necessary enrichment, and minimal toxicity. The addition of whole blood or serum to the basal medium contributes greatly to providing these conditions. Fetal bovine serum is able to support good growth of *H ducreyi*,[16] but in our laboratory we have been using human blood as it is cheaper and readily available from the blood bank.

The characteristic features of *H ducreyi* on solid medium are usually adequate for a presumptive diagnosis. On microscopical examination of young cultures the organism may have wavy parallel rows at the edge of microcolonies. Smears from broth cultures show long chains of cells, which may be arranged in the form of loops. If these features are not seen, microscopical examination may still be useful in excluding the possibility of contamination. Thus, if a uniform suspension is obtained, and most of the cells are isolated Gram-negative bacilli, the organism may not be *H ducreyi* or it may be a mixed culture.

Some uncertainty about the oxidase reactivity of the organism exists. While a negative reaction has been reported by some,[5 6 9] others have reported a positive reaction.[3 16] We found that haemin itself can cause a non-specific reaction and the medium can darken even in the presence of oxidase reagent. If the colonies are too acid the oxidase reaction may be negative, especially with organisms which are weakly positive.[11] By washing the cells first in phosphate-buffered saline pH 7·2, the oxidase reaction was stronger. Nevertheless, further work is necessary to clarify the extent to which haemin may contribute to the reaction. Perhaps the benzidine method of Faller and Schleifer[17] can be used to determine this.

The organism is able to reduce nitrate, though some strains[6 16] have yielded negative results. Initially our strains were incubated for several days so that growth could occur before the test reagents were added. Unpredictable results were obtained with

TABLE *Susceptibility of H ducreyi to antimicrobial agents*

Strains	*β-lactamase-production*	*Minimum inhibitory concentrations (μg/ml)*									
		AMP	*ERY*	*TET*	*STR*	*SLD*	*SXT*	*SPE*	*VAN*	*COL*	*AMP B*
1	+	>8	≤0·5	4	2	16	2	16	>16	4	8
4	+	>8	4	8	4	16	2	4	>16	4	>8
5	+	>8	≤0·5	8	2	8	4	8	>16	8	>8
6	+	>8	1	8	2	8	4	8	16	16	>8
7	+	>8	4	>8	4	>16	8	16	>16	4	>8
9	+	>8	1	4	4	>16	8	16	>16	2	>8
11	+	>8	≤0·5	4	4	>16	4	4	>16	2	>8
12	+	>8	2	>8	4	>16	8	8	>16	4	>8
25	+	>8	≤0·5	>8	16	>16	4	8	>16	4	>8
27	+	>8	≤0·5	>8	4	>16	2	8	>16	16	>8
30	−	0·5	1	>8	8	16	2	8	>16	16	>8
31	+	>8	≤0·5	>8	8	>16	4	4	>16	16	>8
32	+	>8	≤0·5	8	8	>16	4	8	>16	8	>8
54.2	−	0·5	≤0·5	1	2	4	2	8	16	8	>8
C1	−	≤0·25	≤0·5	0·5	8	8	4	16	16	8	>8
C2	−	≤0·25	≤0·5	1	4	4	2	16	>16	4	>8
C3	−	≤0·25	≤0·5	0·5	4	4	2	8	>16	4	>8

+ = Positive, − = negative
AMP = Ampicillin, ERY = erythromycin, TET = tetracycline, STR = streptomycin, SLD = sulphadiazine, SXT = co-trimoxazole, SPE = spectinomycin, VAN = vancomycin, COL = colistin, AMP B = amphotericin B

certain strains. We later found that by reducing the incubation period to six hours all the strains were consistently positive. This was also confirmed by the positive results which were obtained for nitrate reduction using the commercial MICRO-ID system.

Test results for carbohydrate utilisation have been negative[4,9] or equivocal.[16,18] In this study the three hexose sugars—glucose, fructose, and mannose—were consistently utilised by all the strains except CIP 54.2. Compared with the liquid medium systems the filter paper method had better ratios of cells to reagents, and this probably accounted for the more consistent results. Prolonged storage and repeated subculturing may have caused strain CIP 54.2 to lose the ability to utilise the sugars.

The high percentage of strains producing β-lactamase indicates that the use of ampicillin is pointless in Singapore. The enzyme is mediated by a 6·0 megadalton plasmid,[19] which is similar to the TEM-1-type.[20] Some recent evidence, however, suggests that more than one plasmid may be involved.[3] A comparison of MICs of antibiotics for strains from different regions is limited by the difficulty of standardising methodology. Inoculum size and reading criteria will need to be uniform. Our in-vitro results, however, corroborate local clinical experience with various antimicrobial agents; poor results have been obtained with both sulphonamide and tetracycline and good results with streptomycin and co-trimoxazole.[21,22] The value of erythromycin as a therapeutic agent should be explored further in view of its efficacy in vitro and good clinical results.[4]

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Br J Vener Dis 1982;**58**:243-5

Male carriage of Gardnerella vaginalis

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SUMMARY The prevalence of *Gardnerella vaginalis* in the urethra of 430 men attending a clinic for sexually transmitted disease was 11·4%; it was significantly higher in heterosexuals (14·5%) than in homosexuals (4·5%). There was no evidence of rectal or subpreputial carriage of *G vaginalis,* and urethral carriage was not associated with symptoms of urethritis.

Introduction

There is a close association between *Gardnerella vaginalis* and non-specific vaginitis.[1] The disease can be established in women by the inoculation into the vagina of vaginal material from infected women[1] or by the inoculation of pure cultures of *G vaginalis.*[2] There is a high urethral carriage rate of gardnerellas among male partners of infected women,[1,3] and many women whose sexual partners remain untreated become reinfected.[1] These observations suggest that gardnerella-associated vaginitis is a sexually transmitted disease. Little is known, however, about the prevalence of *G vaginalis* in the male population in general and whether it is associated with symptoms.

In this study we have determined the prevalence of urethral carriage of *G vaginalis* in 430 men attending a clinic for sexually transmitted diseases, compared urethral carriage of *G vaginalis* with subpreputial and anorectal carriage, and examined the association between carriage, symptoms of urethritis, and patterns of sexual activity.

Patients and methods

Urethral samples were taken from 430 men attending the Praed Street clinic for sexually transmitted diseases. In addition anorectal samples were taken from 57 of the 430 men and subpreputial samples from 105. Patients were chosen randomly with no attempt at selection on the basis of symptoms. None had received any antimicrobial treatment for at least one month.

CULTURE

The urethral samples, collected with 1-μl disposable inoculating loops, were cultured immediately on to human and horse blood (5% v/v) agar in double-chamber Petri dishes. This enabled any haemolysis produced on the two media to be compared directly. Inoculated plates were stored at 36° in 7% CO$_2$ and transferred to the laboratory within four hours. Anorectal and subpreputial swabs were transported to the laboratory in Amies transport medium, where they were plated on to similar media supplemented with amphotericin B 2 mg/l, gentamicin sulphate 4 mg/l, and nalidixic acid 30 mg/l. All plates were incubated at 36° in 7% CO$_2$ for 48 hours.

Gardnerella vaginalis were identified as catalase and oxidase Gram-variable rods which produced a pronounced diffuse β-haemolysis on human, but little or no haemolysis on horse, blood agar and which fermented starch maltose and glucose.[4]

Results

Urethral samples were obtained from 430 men; *G vaginalis* was isolated from 49, giving a prevalence of 11·4%. Although quantitative cultures were not performed, a heavy growth of *G vaginalis* was obtained in most cases. The double-chamber Petri dishes showed the differential β-haemolysis on horse and human agar very clearly (figure). There was a significant association between the isolation of *G vaginalis* and the absence of symptoms of urethritis (table I). Subpreputial swabs from 105 men and rectal swabs from 57 showed as gardnerellas at either site, although *G vaginalis* colonised the urethra of 13 and four men in the two groups.

The relationship between carriage of *G vaginalis* and patterns of sexual behaviour is shown in table II. Carriage was significantly higher among heterosexuals (14·5%) than among homosexuals (4·5%).

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Accepted for publication 7 March 1982

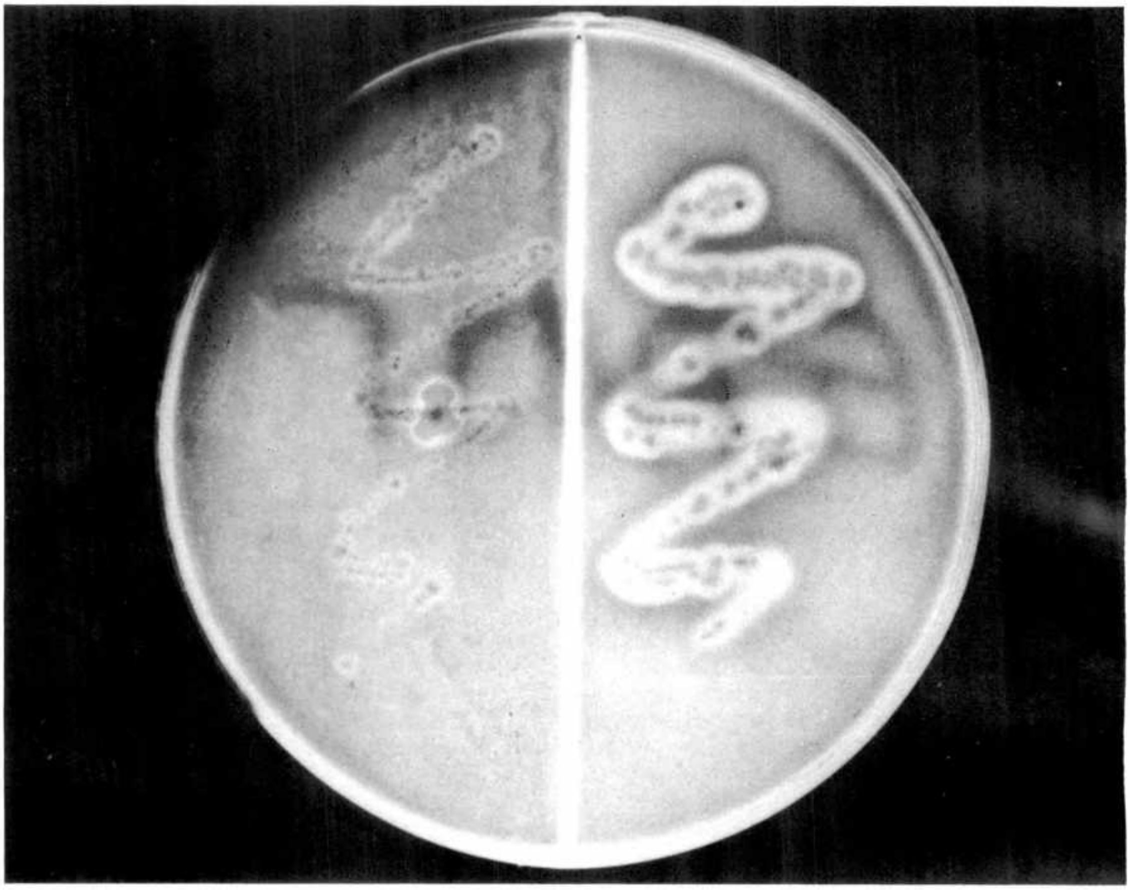

FIGURE *Double-chamber Petri dish containing human blood agar on the right and horse blood agar on the left showing differential haemolysis produced by G vaginalis.*

TABLE I *Relationship between symptoms of urethritis and urethral isolation of G vaginalis*

	Culture results for G vaginalis (% of total)	
Symptoms	*Positive*	*Negative*
Present	16 (7·2)*	206
Absent	33 (15·8)*	175

*$\chi^2 = 8\cdot0$; 0·01<P>0·001

TABLE II *Urethral carriage of G vaginalis in heterosexual, homosexual, and bisexual men*

	Culture results for G vaginalis (% of total)	
	Positive	*Negative*
Heterosexual	42 (14·5)*	246
Homosexual	6 (4·5)*	127
Bisexual	1	8

*$\chi^2 = 9\cdot1$; 0·01<P>0·001

Discussion

Pheifer *et al*[3] found *G vaginalis* in the urethra of 27 of 34 male partners of patients with gardnerella-associated vaginitis. Gardner and Dukes[1] found the organism in 45 of 47 sexual contacts of women infected with *G vaginalis*. Impressive though these figures are, neither study included comparable control groups. Our prevalence of 14·5% among an unselected population of sexually active male heterosexuals and the much lower prevalence among homosexuals does, if generally applicable, strongly support the idea of a male reservoir of *G vaginalis* among the partners of infected women. We are now investigating carriage rates of *G vaginalis* in this highly selected group.

The lack of any association between symptoms and urethral carriage of *G vaginalis* suggests that it is not a primary pathogen. Gardner and Dukes[1] showed that the organism could persist in the male urethra for many months. One case of cystitis apparently caused by *G vaginalis* has been reported.[5]

We found no evidence of rectal carriage of *G vaginalis,* although we have seen this in women. More surprising was our failure to isolate *G vaginalis* from the subpreputial sac, even in those men who were urethral carriers. It is a fastidious organism, and this site may not provide the correct growth conditions for the persistence of *G vaginalis*. Other organisms which colonise the male urethra, such as group B streptococci, can be isolated readily from both the anorectal area and the subpreputial sac.

Anaerobes have been implicated with *G vaginalis* in the pathogenesis of non-specific vaginitis,[3] and recently vibrio-like anaerobes have been isolated in such cases.[6-8] Fontaine *et al*[9] isolated curved anaerobic bacilli from men with non-gonococcal urethritis. We are investigating the relationship between gardnerellas and anaerobes in men and women.

We thank Dr J W R Harris for permission to study patients under his care. This work was supported by a grant from the Medical Research Council.

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Br J Vener Dis 1982;**58**:246-9

Pharmacokinetics of metronidazole and its principal metabolites and their activity against Gardnerella vaginalis

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SUMMARY The hydroxy metabolite of metronidazole was found to be more active against 21 strains of *Gardnerella vaginalis* than the parent compound and less affected by culture in carbon dioxide. After 400 mg oral metronidazole (Flagyl) plasma concentrations of the two agents were below the minimum inhibitory concentrations (MICs) for most *G vaginalis* strains tested. With 2 g metronidazole the plasma concentrations exceeded the MICs of the more sensitive strains. Even with the lower dose of metronidazole clinically useful concentrations of metronidazole and its hydroxy metabolite were present in the urine. Urinary excretion of these compounds may contribute to the efficacy of metronidazole in the treatment of vaginitis associated with *G vaginalis.*

Introduction

Although metronidazole is active against most obligate anaerobes it has little useful clinical activity against facultative bacteria. One exception is *Gardnerella vaginalis,* a facultative Gram-variable bacillus strongly associated with non-specific vaginitis.[1] While metronidazole is only moderately active against *G vaginalis* in vitro (reported minimum inhibitory concentrations (MICs) ranging from 2 mg/l to >50 mg/l) it is very effective in vivo against vaginitis associated with *G vaginalis.*[2 3] Several hypotheses have been put forward to explain this discrepancy. Obligate anaerobes may play some part, indeed possibly the major part, in the aetiology of gardnerella-associated vaginitis, either by providing essential nutrients or by inhibition of host defence mechanisms. The efficacy of metronidazole is, therefore, related to its effect on these organisms rather than on *G vaginalis.*[4] Furthermore, metronidazole may accumulate in the vagina and reach clinically useful concentrations, or the conditions of in-vitro sensitivity testing may not truly reflect those in vivo.[3] Another very attractive theory is based on the finding that the 2-hydroxy-methyl metabolite of metronidazole was very much more active against *G vaginalis* than the parent compound.[5-7] This hydroxy methyl metabolite is a major breakdown product of metronidazole in man and the two compounds have been detected in vaginal secretions.[8]

In this study we have examined the sensitivity of 20 clinical isolates and one stock strain of *G vaginalis* to metronidazole and its two major metabolites in vitro. We have also determined by high-performance liquid chromatography the concentrations of these compounds in the plasma of volunteers after 400-mg and 2-g doses of oral metronidazole and have examined the inhibitory and bactericidal activity of the same plasma samples against *G vaginalis* isolates of varying in-vitro sensitivity.

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Accepted for publication 29 March 1982

Materials and methods

BACTERIA

G vaginalis NCTC 10915 was used as the control strain. Twenty-one strains from patients with vaginitis were isolated on Columbia agar supplemented with human blood (5% v/v) amphotericin B 2 mg/l, gentamicin sulphate 4 mg/l, and nalidixic acid 30 mg/l.[9] They were identified by differential haemolysis (β-haemolysis on human but not on horse blood agar), Gram-stain morphology, oxidase and catalase reactions, and fermentation of starch, maltose, and glucose.[9] Strains were stored in liquid nitrogen.

The activity of the hydroxy metabolite of metronidazole against *Escherichia coli*, *Klebsiella* spp, *Staphylococcus aureus*, and group B streptococci was also investigated.

ANTIMICROBIAL AGENTS

Metronidazole and its 2-hydroxymethyl and 1-acetic acid metabolities were provided as pure powder by May & Baker Ltd.

Minimum inhibitory concentrations (MICs) were determined by the agar dilution techniques using human blood agar and a multipoint inoculator (Denley). The inoculum size was 10^6 colony-forming units (cfu)/ml. Plates were incubated at 37°C for 48 hours, both in 7% CO_2 and anaerobically using the Gaspak system (BBL).

PHARMACOKINETIC STUDIES

Four volunteers were given a single oral dose of 400 mg metronidazole. Plasma concentrations of metronidazole and its two principal metabolites were measured three, six, and 24 hours later and urine concentrations 24 hours later by specific high-performance liquid chromatography.[10] One patient undergoing treatment for giardiasis (metronidazole 2 g daily for three days) also provided blood for plasma concentrations of the three compounds three and 24 hours after each dose.

The inhibitory and bactericidal activity of the plasma samples was determined against six *G vaginalis* isolates of different sensitivity. Doubling dilutions of plasma were made in Isosensitest broth supplemented with 10% heat-inactivated horse serum. The bacterial inoculum was 10^5 cfu/tube. After incubation at 37°C for 48 hours, tubes showing no visible growth were subcultured on human blood agar for a further 48 hours at 37°C in 7% CO_2.

Results

SUSCEPTIBILITY OF *G VAGINALIS* TO METRONIDAZOLE AND ITS METABOLITES

Figs 1 and 2 show the cumulative MICs of metronidazole and its hydroxy metabolite for *G vaginalis* NCTC 10915 and 20 strains grown anaerobically or in 7% CO_2. For all strains the MIC of the acid metabolite was >64 mg/l. The hydroxy metabolite was consistently more active against *G vaginalis* than metronidazole itself and its activity less affected by culture in CO_2 rather than by anaerobic culture.

Table I shows that the hydroxy metabolite of metronidazole was not active against *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, or group B streptococci.

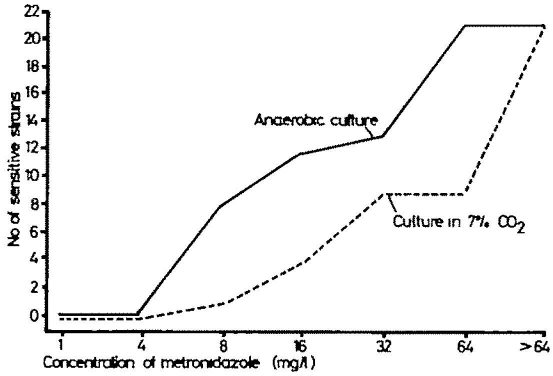

FIG 1 *Cumulative MIC of metronidazole for 21 isolates of G vaginalis cultured anaerobically and in 7% CO_2.*

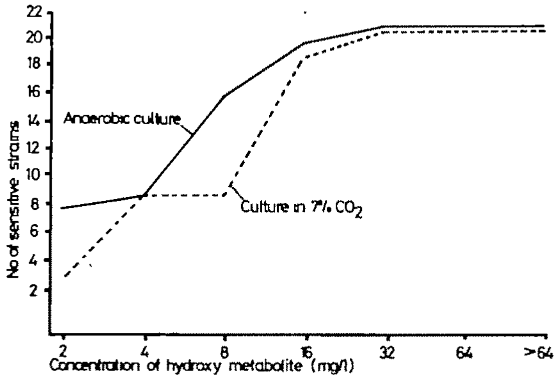

FIG 2 *Cumulative MIC of hydroxy metabolite of metronidazole for 21 isolates of G vaginalis cultured anaerobically and in 7% CO_2.*

TABLE I *Susceptibility of facultative bacteria to metronidazole and hydroxy metabolite*

	Minimum inhibitory concentration (mg/l)			
	Metronidazole		*Hydroxy metabolite*	
Organism	*AnO_2*	*CO_2*	*AnO_2*	*CO_2*
Group B streptococcus	>256	>256	>256	>256
Staphylococcus aureus	>256	>256	>256	>256
Escherichia coli	128	>256	128	>256
Klebsiella aerogenes	128	>256	128	>256

AnO_2 = anaerobic

PLASMA AND URINARY CONCENTRATIONS

Four volunteers each received 400 mg metronidazole orally. Plasma concentrations of metronidazole and its hydroxy metabolite three, six, and 24 hours later are shown in table II. No acid metabolite was detected in any of the plasma samples. The plasma

TABLE II *Plasma concentrations of metronidazole and its hydroxy metabolite in four subjects three, six, and 24 hours after 400 mg oral metronidazole. (No acid metabolite detected.)*

	Plasma concentrations (mg/l) at (hours):					
	Metronidazole			*Hydroxy metabolite*		
Subject No	*3*	*6*	*24*	*3*	*6*	*24*
1	7·0	5.2	1·0	1·0	1·3	0·7
2	8·1	6·0	1·1	0·7	0·8	0·8
3	8·3	6·4	1·3	0·6	0·9	0·7
4	13·0	9·6		1·0	1·4	

concentration of metronidazole fell steadily over 24 hours, but the hydroxy metabolite remained roughly constant. At three hours the hydroxy metabolite represented 9% of the total drug; at 24 hours it represented nearly 40%. The urinary concentrations of the parent compound and its major metabolites in samples obtained 24 hours after are shown in table III. The urinary concentrations of the hydroxy and acid metabolites were greater than those of metronidazole.

Table IV shows the plasma concentrations over four days in a patient taking 2 g metronidazole a day orally for three days. No acid metabolite was detected in the plasma. Three hours after the antibiotic had been taken plasma concentrations of metronidazole were between 47 mg/l and 50 mg/l falling to between 10·2 mg/l and 12·7 mg/l by 24 hours. The plasma concentration of hydroxy metabolite, although much lower than that of metronidazole, showed little variation between the concentrations at three hours and 24 hours. Multiple dosages gave rise to a steady increase in metabolite concentrations over four days, comprising about 10% of total drug at three hours rising to about 30% at 24 hours. These results parallel those seen after a 400-mg dose of metronidazole.

TABLE III *Urine concentrations of metronidazole and its principal metabolites 24 hours after 400 mg oral metronidazole*

	Urine concentrations (mg/l)		
Subject No	*Metronidazole*	*Hydroxy metabolite*	*Acid metabolite*
1	*4·3*	*10·8*	*6·0*
2	*7·9*	*48·0*	*33·6*
3	*6·1*	*35·0*	*24·4*
4	*7·3*	*38·0*	*57·0*

TABLE IV *Plasma concentrations of metronidazole and its hydroxy metabolite during a three-day course of oral metronidazole 2 g daily. (No acid metabolite detected.)*

	Plasma concentrations (mg/l)	
Day	*Metronidazole*	*Hydroxy metabolite*
1		
Three hours after antibiotic	50·4	4·3
2		
Before antibiotic	12·7	4·5
Three hours after antibiotic	48·4	6·8
3		
Before antibiotic	10·2	5·0
Three hours after antibiotic	47·0	8·1
4	11·8	6·5

INHIBITORY AND BACTERICIDAL EFFECTS ON *G VAGINALIS*

None of the plasma samples taken after the 400-mg dose of metronidazole either killed or inhibited the growth of any of the *G vaginalis* isolates or strains tested. Plasma samples taken three hours after the 2-g dose inhibited and killed two isolates, with MICs of 2 mg/l for the hydroxy metabolite and 8 mg/l for metronidazole, at a dilution of 1/8. Plasma taken 24 hours after the 2-g dose killed one of these two isolates at a dilution of 1/8 but neither inhibited nor killed the other strain. The other four strains, including the NCTC control, were unaffected by any of the plasma samples.

Agglutination was seen with higher concentrations of some plasma samples and *G vaginalis*.

Discussion

We agree with other workers,[5-7] who found that while metronidazole was moderately active against *G vaginalis* its hydroxy metabolite was far more so. Gardnerellas are alone among facultative bacteria in being so sensitive to these agents. Whether *G vaginalis* shares with obligate anaerobes the capacity to reduce metronidazole[11] and whether the greater activity of the hydroxy metabolite is related to greater uptake or more effective killing remains to be determined. It does seem likely that the greater activity of the hydroxy metabolite against *G vaginalis* is specific for this organism as we did not find it to be any more active than metronidazole against other facultative bacteria. Ralph and Amatnieks[5] found it to be less active than metronidazole against *Bacteroides* spp.

MICs of metronidazole were generally lower when tested anaerobically than in CO$_2$ but this difference was less noticeable with the hydroxy metabolite. Our MICs are somewhat higher than those reported by Ralph and Amatnieks.[5]

Three hours after ingestion the metabolite accounted for about 10% of total drug in the plasma rising to 30-40% at 24 hours. This increase in the proportion of the metabolite compared with a steady fall in the concentration of metronidazole over the same time.[10] There was some evidence of a steady rise in concentrations of hydroxy metabolite in the patient receiving metronidazole for three days. The hydroxy metabolite was the major product in the urine, with high concentrations being reached 24 hours after a 400-mg dose of metronidazole. After 400 mg metronidazole the plasma concentrations of both compounds were lower than the MICs for most *G vaginalis* strains. With the 2-g dose, however, plasma concentrations of these agents were above the MICs for the more sensitive strains. The urine concentrations of the hydroxy metabolite were far in excess of the MICs of most strains tested. The effect of plasma samples on *G vaginalis* was that expected from these results. It is possible that a five-day course of metronidazole 400 mg twice daily might have a cumulative effect resulting in concentrations rather higher than we obtained. If the sensitivities of our 20 clinical isolates are representative and if the concentrations in vaginal secretions equal those in serum, however, it must be doubtful whether this would be effective directly against many strains of *G vaginalis*.

A treatment regimen based on 500 mg metronidazole twice daily does appear to be effective in *G vaginalis*-associated vaginitis.[4] If *G vaginalis* is the primary pathogen and if the success of metronidazole treatment is not based on its effect on accompanying anaerobes, how then can this success be explained? There are three possibilities. Firstly, the antibiotic could be concentrated within vaginal secretions to a much higher degree than in serum. Secondly, antibiotic excreted in the urine may play a major part in killing vaginal pathogens. Even 24 hours after a single 400-mg dose of metronidazole the urinary concentrations of hydroxy metabolite in three of the four subjects were above the MICs of all *G vaginalis* strains tested. The fourth subject had lower concentrations, but as total urine volumes were not measured the amount excreted is not known. Thirdly, concentrations of antibiotic below the MIC may still affect the pathogenesis of the infective process.

G vaginalis appears to be primarily a pathogen of mucosal surfaces. Adhesion to vaginal epithelium is likely to play an important part in the infection; indeed, the clue cell, an epithelial cell covered with adherent gardnerellas, is a diagnostic feature of the condition. Relatively low concentrations of metronidazole or its hydroxy metabolite could affect adherence to cells. A similar phenomenon has been observed with other bacteria[12] and deserves further investigation. Although we have not measured drug concentrations after an 800-mg dose of metronidazole, it is likely that those reached on a regimen of 800 mg twice daily would directly inhibit many *G vaginalis* strains.

The higher dosage of 2 g does appear to give serum concentrations of the hydroxy metabolite which are directly active against many strains of *G vaginalis*. Single-dose treatment is more acceptable to patients because of the bitter taste of metronidazole and its possible effects when combined with alcohol.

Urethral carriage of *G vaginalis* in male partners may be important in the epidemiology of vaginitis.[13] The high concentrations of hydroxy metabolite in the urine suggest that metronidazole should be very effective in eradicating such carriage possibly after only one or two doses.

We thank Mr M G Sankey and Mrs J E Holt for excellent technical assistance.

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Isoenzyme characterisation of Trichomonas vaginalis

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SUMMARY Clones of 32 strains of *Trichomonas vaginalis* isolated from patients attending a venereal diseases clinic were compared among themselves and with authentic *Pentatrichomonas hominis* on the basis of their isoenzyme patterns for eight enzymes by thin-layer starch-gel electrophoresis. The enzymes examined were: glucose phosphate isomerase (GPI); phosphoglucomutase (PGM); malic enzyme (NADP$^+$) (ME); hexokinase (HK); malate dehydrogenase (NAD$^+$) (MDH); glucose-6-phosphate dehydrogenase (G6PD); aldolase (ALD); and lactate dehydrogenase (LDH).

From the isoenzyme patterns of four enzymes (LDH, MDH, HK, and GPI) the strains of *T vaginalis* could be divided clearly into five groups. PGM showed differences in only one strain, while two other enzyme patterns (ME and ALD) were the same for all the strains of *T vaginalis* tested. All isolates were clearly distinguishable from *P hominis*. Although G6PD patterns were not sharp some differences were evident among *T vaginalis* strains.

Introduction

Teras[1] claimed that there was reason to believe that the human genitourinary trichomonad consisted of different types (possibly even different subspecies), on which depended the pathology and clinical course of trichomoniasis. A variety of immunological, serological, and pathological tests have been used to try to distinguish between different strains of *Trichomonas vaginalis.*[1-7] Although during the last few years some differences have been defined, very few data are available on the intraspecific variation in the biochemical activity of trichomonads and the relationship of these varieties to other biological properties. Isoenzyme techniques for the identification of isolates of parasitic protozoa, such as *Leishmania, Trypanosoma, Entamoeba, Plasmodium,* and others, have become essential in understanding the epidemiology of parasitic diseases, and there can be no doubt that biochemical analysis constitutes a fruitful area for future studies.

The aim of this work was to apply the technique of isoenzyme analysis (by thin-layer starch-gel electrophoresis) to the study of *T vaginalis.*

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Accepted for publication 11 February 1982

Materials and methods

ORGANISMS

Specimens of vaginal fluid were collected, by a method similar to that of Robertson *et al,*[8] from female patients attending the venereal diseases clinic, Middlesex Hospital. Two mouse-virulent strains of *T vaginalis* were obtained from Italy by courtesy of Professor P Cappuccinelli; they were strain 1 (cryopreserved in London as LUMP 1192) and strain a20 (LUMP 1193). *Pentatrichomonas hominis* was obtained from the American type culture collection (ATCC 30000).

CULTIVATION

Human *T vaginalis* strains and the mouse-virulent strains were grown in the medium of Lumsden *et al,*[9] modified[10] by the replacement of liver digest by neutralised liver digest (Oxoid) and by altering the pH of the buffer component to 6·8. *P hominis* was grown in Stenton's[11] medium.

CLONING

Suspensions of cultured *T vaginalis* organisms were diluted and pour plates prepared by the method of Hollander.[12] The medium used was the TYM medium of Diamond,[13] modified by Hollander.[14] Dilutions were calculated to give 15-30 colonies per plate; plating efficiency was about 80%. Single colonies were picked up and cultured further in liquid media.

PREPARATION OF EXTRACTS FOR ELECTROPHORESIS

The method used was basically that of Godfrey and Kilgour.[15] Organisms were harvested in the late exponential growth phase by centrifugation at 1000 × *g* for 30 minutes at 4°C and washed three times in phosphate-buffered saline. The washed pellets were lysed by mixing them with an equal volume of a hypotonic solution containing 1·0 mmol/l EDTA, 1·0 mmol/l dithiothreitol, and 1·0 mmol/l ε-aminocaproic acid and freezing. They were kept overnight at −20°C, thawed, and then centrifuged at 34 000 × *g* for 45 minutes at 4°C. The clear supernatant was removed and placed on ice; it was frozen as rapidly as possible in beads at −190°C until required or used immediately for electrophoresis.

ELECTROPHORESIS AND ENZYME STAINING

Electrophoresis was carried out as described by Wraxall and Culliford[16] and by Kilgour and Godfrey.[17] Modifications were made to improve the resolution of the system for use with trichomonad parasites. After electrophoresis the enzymes were visualised using formazan development in agar overlays by similar staining methods to Bagester and Barr[18]; controls were included for each enzyme by omitting the specific substrate. Substrates and developing reagents were obtained from Sigma. The conditions used in electrophoresis and the location of these enzymes are summarised in tables I and II. Soluble extracts from *T vaginalis* strains and other trichomonads were specifically stained for the following enzymes: (a) aldolase (ALD) E.C.2.4.1.13; (b) malic enzyme (oxaloacetate decarboxylating) (ME) E.C.1.1.40; (c) lactate dehydrogenase (LDH) E.C.1.1.1.27; (d) glucose phosphate isomerase (GPI) E.C.3.5.1.9; (e) phosphoglucomutase (PGM) E.C.2.7.1.1; (f) malate dehydrogenase (NAD^+ oxireductase) (MDH) E.C.1.1.1.37; (g) hexokinase (HK) E.C.2.7.1.1; and (h) glucose-6-phosphate dehydrogenase (G6PD) E.C.1.1.1.49.

Results

The results of electrophoresis of the enzymes examined are summarised in fig 1 and shown in fig 2. In most cases the enzyme patterns appeared within five minutes after specific staining and rapidly became diffuse on further incubation. The enzyme patterns were classified into two groups.

ENZYMES WITH SIMILAR ELECTROPHORETIC PATTERNS

Aldolase

All extracts of *T vaginalis* showed five separate, sharp, intense bands (figs 1*a* and 2*a*). The pattern of this enzyme was found to be constant, even when the electrophoretic conditions were slightly changed. The mouse-virulent strains showed the same patterns as normal *T vaginalis*.

Malic enzyme

The electrophoretic pattern of different strains of *T vaginalis* and the mouse-virulent strains can be seen in fig 2*b* and are summarised in fig 1*b*. Each extract showed three bands with a weak (fastest) one, which

TABLE I *Optimum conditions* for enzyme electrophoresis*

Enzyme	*Tank buffer (1 litre)*	*pH*	*Volts/cm across gel*	*Current (mA)*	*Gel buffer made from tank buffer*	*Running time (hours)*
GPI	0·122 mol/l Na_2HPO_4/0·078 mol/l NaH_2PO_4	7·0	16	15	1/13	2·5
PGM†	0·1 mol/l Tris/0·1 mol/l maleic/0·01 mol/l EDTA/ 0·01 mol/l $MgCl_2$	7·4	16	15	1/10	2·5
ME	0·122 mol/l Na_2HPO_4/0·078 mol/l NaH_2PO_4	7·0	16	15	1/10	3
HK	Stock: 0·45 mol/l Tris/0·01 mol/l EDTA/ 0·1 mol/l boric acid/0·0125 mol/l $MgCl_2$ Tank: dilute 1/7	8·9	20	20	1/10	3·5
MDH	0·05 mol/l phosphate/0·007 mol/l citric acid	7·0	20	20	1/13	3
G6PD	0·122 mol/l Na_2HPO_4/0·078 mol/l NaH_2PO_4	7·0	16	15	1/10	3
ALD	0·08 mol/l Na_2HPO_4/0·019 mol/l NaH_2PO_4	7·4	20	20	1/10	3·5
LDH†	0·1 mol/l Tris/0·1 mol/l maleic/0·01 mol/l EDTA/ 0·01 mol/l $MgCl_2$	7·8	20	20	1/5	3

*Temperature of the cooling plate was kept at 4°C throughout the run

†With reference to electrode buffers and PGM and LDH, the Tris, EDTA, and maleic acid components were first neutralised with NaOH in solution after which the $MgCl_2$ was added and the pH further adjusted with NaOH to the required value.

GPI = glucose phosphate isomerase; PGM = phosphoglucomutase; ME = malic enzyme; HK = hexokinase; MDH = malate dehydrogenase; G6PD = glucose-6-phosphate dehydrogenase; ALD = aldolase; LDH = lactate dehydrogenase; EDTA = ethylenediamine tetra acetic acid.

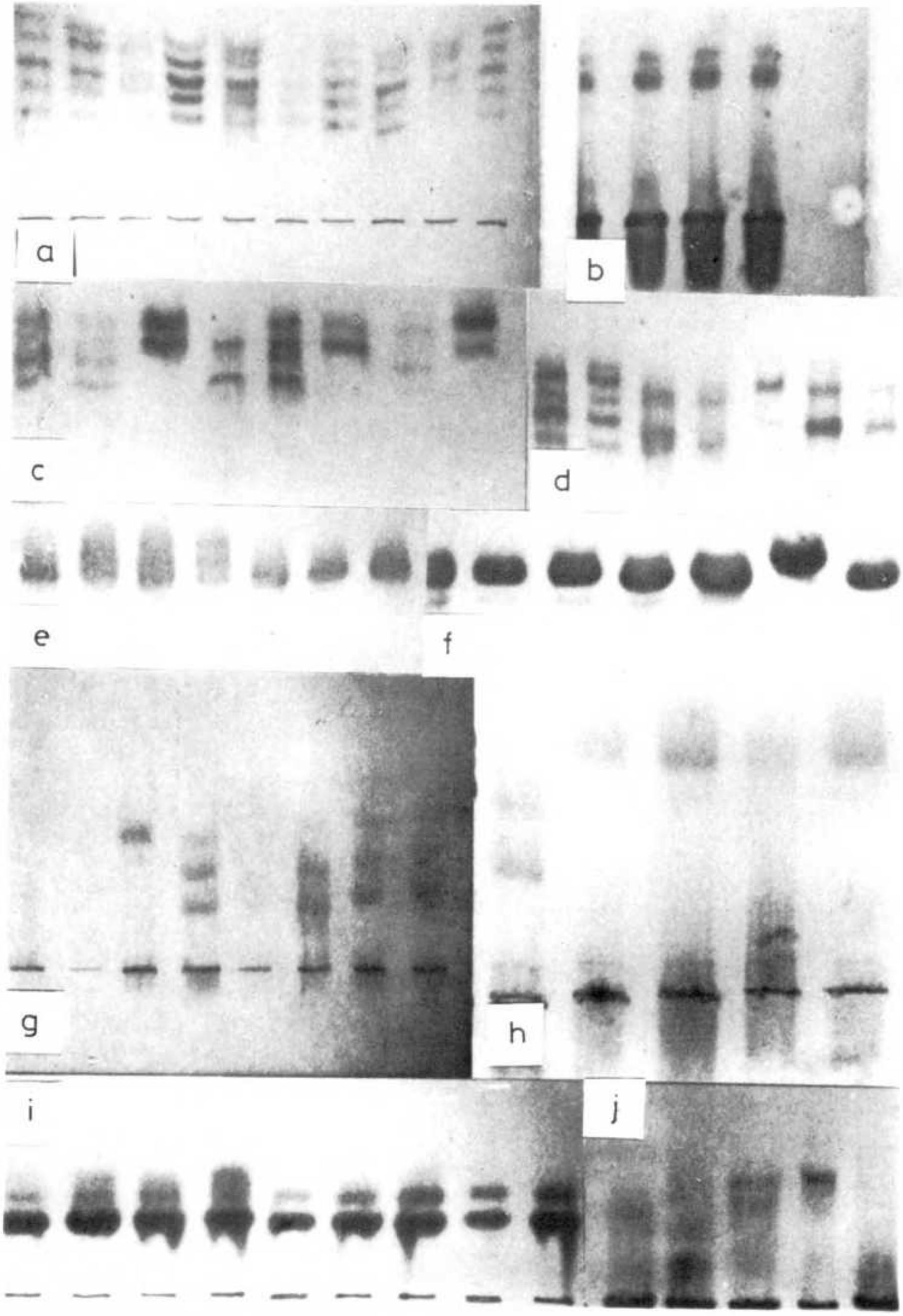


FIG 2 *Photographs of isoenzyme patterns (zymodemes) for the eight enzymes; (a) aldolase (ALD); (b) malic enzyme (ME) (oxaloacetate decarboxylating); (c) and (d) lactate dehydrogenase (LDH); (e) glucose phosphate isomerase (GPI); (f) phosphoglucomutase (PGM); (g) and (h) malate dehydrogenase (MDH); (i) hexokinase (HK); (j) glucose-6-phosphate dehydrogenase (G6PD).*

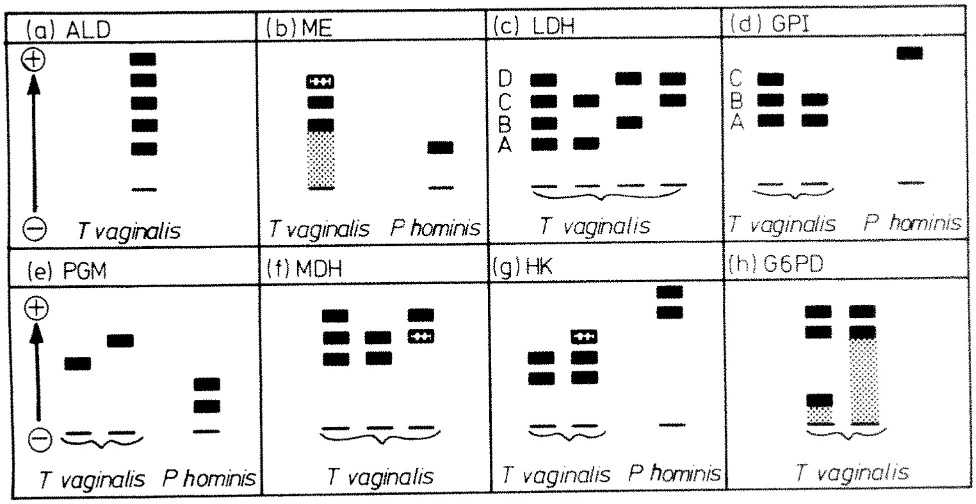


FIG 1 *Diagrams summarising enzyme electrophoretic patterns for: (a) aldolase (ALD) of various T vaginalis strains; (b) ME (malic enzyme "oxaloacetate decarboxylating") of various T vaginalis strains and P hominis; (c) lactate dehydrogenase (LDH) of various T vaginalis strains; (d) glucose phosphate isomerase (GPI) of various T vaginalis strains and P hominis; (e) phosphoglucomutase (PGM) of various T vaginalis strains and P hominis; (f) malate dehydrogenase (NAD$^+$ oxireductase) (MDH) of various T vaginalis strains; (g) hexokinase (HK) of various T vaginalis strains and P hominis; (h) glucose-6-phosphate dehydrogenase (G6PD) of various T vaginalis strains.*

developed later than the other two. Diffusion from the origin is characteristic of zymograms of this particular enzyme.

ENZYMES WITH DIFFERENT PATTERNS

Lactate dehydrogenase

Four different patterns among different extracts of *T vaginalis* were observed (figs 1*c*, 2*c*, and 2*d)*; LDH I—pattern with four regular, sharp, intense bands (A, B, C and D) (samples 1 and 2 in fig 2*c* and 2*d*); LDH II—pattern with two sharp intense bands corresponding to bands A and C in pattern I (sample 4 in fig 2*c* and samples 3 and 4 in fig 2*d*); LDH III—pattern with two sharp intense bands corresponding to bands B and D in pattern I (fig 2*d*, samples 5, 6, and 7); LDH IV—pattern with two sharp intense bands corresponding to bands C and D in pattern I (fig 2*c*, samples 3, 6, 8).

Glucose phosphate isomerase (GPI)

T vaginalis strains usually gave patterns with three diffuse bands (A, B, and C; samples 2, 3, and 4 in fig 2*e*) or two bands (A and B; samples 5, 6, and 7 in fig 2*e*). One extract (mouse-virulent strain LUMP 1192), however, showed band A missing, while one other strain (LUMP 1242) showed an extra slow band.

Phosphoglucomutase

Almost all extracts of *T vaginalis* strains showed the same electrophoretic pattern with a single band, which was always in the same position and diffused within five minutes after development (figs 1*e* and 2*f*). Only one extract (mouse-virulent strain LUMP 1192) gave a band relatively faster than all the others (sample 6 in fig 2*f*).

Malate dehydrogenase

T vaginalis strains showed three different patterns (figs 1*f*, 2*g*, and 2*h*): MDH I—pattern with three bands (A, B, and C) (sample 4 in fig 2*g*); MDH II—pattern with two bands (as A and B in pattern I) (sample 6 in fig 2*g*); MDH III—pattern with two bands (as B and C in pattern I) (samples 2, 3, 4, and 5 in fig 2*h*). One of the two Italian mouse-virulent strains (LUMP 1192) showed a MDH III pattern while the other (LUMP 1193) showed a MDH I pattern.

Hexokinase (HK)

Two electrophoretic patterns were found (figs 1*g* and 2*i*): HK I—pattern with two intense bands (A and B) and a third weak band (C) (samples 2, 3, and 4 in fig 2*i*); HK II—pattern with two intense bands corresponding to bands A and B in HK I pattern but no band C (samples 5, 6, 7, 8, and 9 in fig 2*i*). The mouse-virulent strain, LUMP 1192, showed the HK I pattern while LUMP 1193 showed the HK II pattern.

TABLE II *Composition of enzyme developers*

	Developing conditions							
*Enzyme**	*Buffer*	*Substrate*	*Coenzyme*	*Other conditions*	*$MgCl_2$*	*MTT*	*PMS*	*Agar L28 (12 mg/ml)*
GPI	0·3 mol/l Tris/HCl, pH 8, 10 ml	10 mg/ml, F6P 1 ml	10 mg/ml NADP+ (4-Na-salt), 0·5 ml	100 U/ml G6PD solution, 500 µl	0·5 ml	2 mg	1 mg	10 ml
PGM	0·3 mol/l Tris/HCl, pH 7·4, 10 ml	25 mg G1P with 1% G1,6DP	10 mg/ml NADP+ (4-Na-salt), 0·4 ml	100 U/ml G6PD solution, 150 µl	0·5 ml	2 mg	1 mg	10 ml
HK	0·1 mol/l Tris/HCl, pH 7·4, 10 ml	450 mg D-glucose, 60 mg $ATP(Na)_2$	10 mg/ml NADP+ (4-Na-salt), 1·0 ml	100 U/ml G6PD solution, 10 µl	0·5 ml	4 mg	2 mg	10 ml
ME	0·3 mol/l Tris/HCl, pH 7·4, 8 ml	Malate solution† 1 ml	10 mg/ml NADP+ (4-Na-salt), 0·5 ml		2 ml	5 mg	2 mg	10 ml
MDH	0·3 mol/l Tris/HCl, pH 8, 6 ml	Malate solution† 4 ml	10 mg/ml NAD+ (di-Na-salt), 1 ml			2 mg	1 mg	10 ml
LDH	0·05 mol/l Tris/HCl, pH 8, 10 ml	Ca-lactate (Sigma) 35 mg	10 mg/ml NAD+ (di-Na-salt), 2·5 ml			5 mg	2 mg	10 ml
ALD	0·3 mol/l Tris/HCl, pH 8, 6 ml	F1,6DP 72 mg, glyceraldehyde-3-phosphate dehydrogenase 96 units	10 mg/ml NAD+ (di-Na-salt), 0·72 ml	0·3 mol/l Na-arsenate 1 ml; H_2O 2 ml		2 mg	1 mg	10 ml
G6PD	0·2 mol/l Tris/HCl, pH 8, 7 ml	G6P 5 mg	10 mg/ml NADP+ (4-Na-salt), 0·2 ml		3 ml	2 mg	1 mg	10 ml

*For full name, see table I

†10 mg/ml L-malic acid adjusted to pH 7·0 with 1·0 mol/l NaOH

F6P = fructose 6 phosphate; G1P = glucose 1 phosphate; G1,6DP = glucose 1,6 diphosphate; ATP $(Na)_2$ = disodium salt of adenosine 5'triphosphate; F1,6DP = fructose 1,6 diphosphate; G6P = glucose-6-phosphate; MTT = 3(4,5 dimethylthiazol-2-γl) 2,5 diphenyltetrazolium bromide; PMS = phenazine methosulphate.

Glucose-6-phosphate dehydrogenase (G6PD)

The results with this enzyme gave some indication of two different types of electrophoretic patterns (figs 1*h* and 2*j*); however, as the bands tended to be very diffuse they were not included in the final classification. The electrophoretic patterns for the enzymes of *P hominis* tested are different from the other trichomonads and can be seen in fig 1*b, d, e,* and *g*). Using the enzyme patterns of LDH, MDH, HK, and GPI the *T vaginalis* may be divided into five groups (zymodemes) (fig 3 and table III).

TABLE III *Distribution of isolates* of T vaginalis among the five isoenzyme groups (zymodemes) defined in this study*

Group	*T vaginalis strain*
I	75/7479; 75/8040; 75/8095; 75/8555; 76/415; 76/2002; 76/7689; LUMP 1165; LUMP 1193; LUMP 1314
II	76/434; 76/991; LUMP 1031; LUMP 1042; LUMP 1043; LUMP 1315
III	75/8213; 76/714; LUMP 994; LUMP 1044; LUMP 1160; LUMP 1264
IV	75/2040; 75/8403; LUMP 1064; LUMP 1065
V	76/754; LUMP 996; LUMP 1046; LUMP 1232

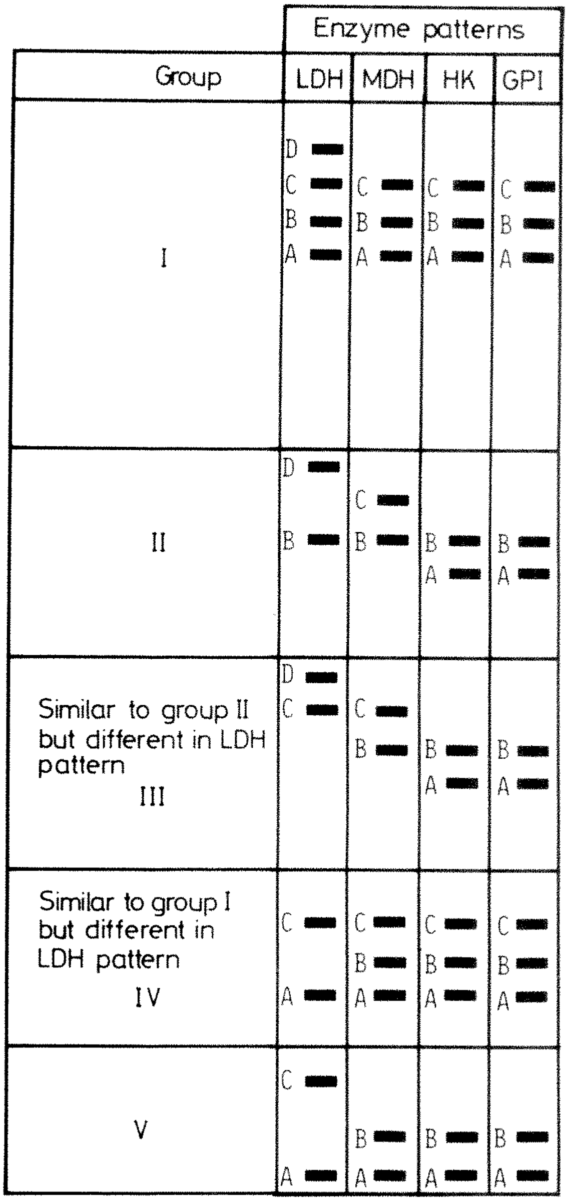
*Of the 32 isolates examined, two (LUMP 1192 and 1242) were not assigned owing to anomalous patterns with GPI (both) and PGM (LUMP 1192).

Discussion

In the present study isoenzyme analysis by thin-layer starch-gel electrophoresis was a promising method for the characterisation of trichomonads. It is a relatively simple technique and highly reproducible. It is important, however, that the isoenzyme patterns of several enzymes are examined when different strains of different trichomonads are being compared. In general, the isoenzyme patterns of all enzymes used in this study showed considerable differences between different trichomonad species when these were examined (unpublished data).

The fact that all strains of *T vaginalis* showed similar isoenzyme patterns of certain enzymes, for example, aldolase (ALD) and malic enzyme (ME), should not be regarded as a disadvantage; rather it tends to give confidence in the validity of the species.

At first the isoenzyme patterns of phosphoglucomutase (PGM) appeared to be similar in all *T vaginalis* strains tested; however, one of the two Italian strains (1192) showed a faster band. It is, therefore, possible that the enzyme will vary between strains. From the work done so far we cannot explain why some strains failed to show any bands at all in some enzymes. This could have been due to lack of activity, unsatisfactory conditions for the development, or because in *T vaginalis* and other trichomonads tested the enzyme is unusually labile. Another possibility is that the relatively crude extracts used might not be suitable for testing such enzymes. Our results with glucose-6-phosphate dehydrogenase would support the latter suggestion.



Group	Enzyme patterns			
	LDH	MDH	HK	GPI
I	D ▬ C ▬ B ▬ A ▬	C ▬ B ▬ A ▬	C ▬ B ▬ A ▬	C ▬ B ▬ A ▬
II	D ▬ B ▬	C ▬ B ▬	B ▬ A ▬	B ▬ A ▬
Similar to group II but different in LDH pattern III	D ▬ C ▬	C ▬ B ▬	B ▬ A ▬	B ▬ A ▬
Similar to group I but different in LDH pattern IV	C ▬ A ▬	C ▬ B ▬ A ▬	C ▬ B ▬ A ▬	C ▬ B ▬ A ▬
V	C ▬ A ▬	B ▬ A ▬	B ▬ A ▬	B ▬ A ▬

FIG 3 *Summary of the electrophoresis patterns found among T vaginalis isolates, which divide the organism into five groups (zymodemes).*

With this enzyme, while some strains of *T vaginalis* showed diffuse patterns, many others did not show any bands at all.

Among eight enzymes found to be satisfactory in the present study, lactate dehydrogenase (LDH) seemed to be very promising. The four different isoenzyme patterns (I, II, III, and IV) with this enzyme divided the *T vaginalis* strains into four groups. Interestingly, Teras and his collaborators[2] in Estonia divided his isolates also into four groups on the basis of antigenic type; it has not yet proved possible to see if the two classifications coincide.

With glucose phosphate isomerase (GPI), the electrophoretic patterns from most strains of *T vaginalis* fell into two groups. There is a possibility, however, of two further groups existing, because one of the (Italian) strains (1192) showed a missing band and another (LUMP 1242) showed an extra slower band which was not present in the other groups. Malate dehydrogenase (NAD$^+$ oxireductase) (MDH) is another enzyme which showed promising and reproducible results. *T vaginalis* strains could be divided into three different groups according to the electrophoretic pattern when using this enzyme. Possibly, more variability exists, because two forms (isoenzymes) of malate dehydrogenase (MDH) of one strain of *T vaginalis* were isolated by Brugerrolle *et al*[19] using sucrose density gradient and polyacrylamide gel electrophoresis. One form was located in the soluble fraction and the other was linked to the cytoplasmic "dense granules". Although these workers[19] have not compared different *T vaginalis* strains, their electrophoretic patterns of the soluble form of MDH were similar to one of the three patterns we found.

Using hexokinase (HK) enzyme we obtained two different electrophoretic patterns which divided *T vaginalis* strains into two groups. Comparison of these two groups with the three groups obtained by Teras *et al*[20] is difficult at this stage because they classified *T vaginalis* strains according to the total activity of the enzyme. Using this method of grouping they were able to correlate their results with virulence but not with antigenic differences among the 25 strains they used.

In the present study, using the enzyme patterns of LDH, MDH, HK, and GPI, *T vaginalis* was divided into five groups (zymodemes). While the importance of the present work cannot be wholly assessed until more is known about other properties which differ between zymodemes, at least there are now markers available to identify subpopulations of *T vaginalis* and distinguish them from other trichomonads. The enzymes tested in this study are unlikely to be the only ones suitable, and others can be looked for with some confidence. This method of identification offers the beginning of a biochemical classification to supplement the present morphological, immunological, and pathological classifications.

We thank Miss S M Lanham, Dr D G Godfrey, Dr V Kilgour, and Mr J E Williams for a great deal of help and advice with the enzyme electrophoresis; the staff of James Pringle House for all their help in obtaining specimens; and the Egyptian Government for financial support to M A S.

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Br J Vener Dis 1982;58:257-8

Treatment of trichomoniasis with metronidazole rectal suppositories

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SUMMARY Since a single dose of metronidazole in suppository form is very effective in the prevention of postoperative Gram-negative anaerobic infections, 84 patients with vaginal trichomoniasis were treated with metronidazole suppositories (two 1-g suppositories in a single dose). The cure rate in this series was 94%.

Introduction

Metronidazole is the drug of choice in the treatment of trichomoniasis. A single oral dose of 2 g metronidazole achieves a high cure rate.[1 2] The use of metronidazole suppositories is useful in the prevention of postoperative infections.[3 4] Within two hours of insertion into the rectum 2 g metronidazole in suppository form produces a serum concentration of 4 µg/ml.[5 6] After reaching a peak concentration of 12-20 µg/ml in about eight hours, the serum concentration of metronidazole falls slowly to about 4 µg/ml in 24 hours.[7] Since in-vitro tests have shown that most strains of *Trichomonas vaginalis* are killed when the concentration of metronidazole in the surrounding medium is between 0·25 µg/ml and 1·25 µg/ml, a 2-g rectal dose should be effective in the treatment of trichomoniasis. As a prolonged course of any medication results in poor patient compliance, treatment with a suppository which is completed in the clinic may be invaluable in eradicating vaginal trichomoniasis.

Patients and methods

Eighty-four women with vaginal trichomoniasis attending the department of genitourinary medicine from May 1980 to August 1981 were studied. Patients included in the project were unselected. The appropriate microbiological investigations were undertaken to confirm or exclude the presence of gonorrhoea, syphilis, candidosis, chlamydial infection, and *Herpesvirus hominis* infection. Cervical cytology was performed routinely. Vaginal secretions were examined by direct microscopy and culture for infection with *T vaginalis*.

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Accepted for publication 3 March 1982

TREATMENT

When trichomoniasis had been diagnosed, the women were treated with metronidazole suppositories. A lubricated plastic proctoscope was inserted into the rectum, through which the suppositories (total dose of 2 g metronidazole) were introduced. The patients were asked to refrain from sexual activity for at least one week.

FOLLOW UP

The patients were examined at one week and four weeks after treatment. At the follow-up examinations, microscopy and cultures for *T vaginalis* and *C albicans* were performed and other investigations undertaken when necessary. The women were asked leading questions about the side effects and the acceptability of the treatment. The patients who had been reinfected were treated with 2 g metronidazole suppositories and those who were regarded as treatment failures were given 200 mg metronidazole orally three times a day for seven days.

SEXUAL CONTACTS

Forty-nine men who were named contacts of the women in the study attended the department and were treated empirically with 200 mg metronidazole orally three times daily for seven days. The female patients were asked not to take any of the tablets which had been given to their consorts.

Results

Trichomoniasis was diagnosed by direct microscopy in three (3·6%) women, by culture in four (4·8%), and by both microscopy and culture in 77 (91·7%).

The mean age of the patients was 23·1 (range 14-49) years; 12 were married; 28 used oral contraception, 44 took no contraceptive precautions, and the remainder used other methods.

Concomitant infection with gonorrhoea was diagnosed in 24% of these women; 19% had candidosis, 7% had genital herpes, and 6% had genital warts.

TREATMENT

The results of treatment with metronidazole suppositories are shown in table I; five patients did not return for follow up one week after treatment had been given.

TABLE I *Results of treatment of trichomoniasis with metronidazole suppositories at follow up seven days after treatment*

Total No of patients	*Primary failure (%)*	*Reinfected (%)*	*Cured (%)*
79	5 (6)	6 (7)	74 (94)

Reinfection occurred in patients who had had sexual intercourse within seven days of treatment either with a new partner or with an untreated regular partner. All six of these women were treated successfully with a second dose of rectal metronidazole.

The five women who were considered to have had primary failure of the drug regimen were treated with oral metronidazole; this was not successful in one woman. The strain of *T vaginalis* isolated from her vagina had a minimum inhibitory concentration (assessed under anaerobic conditions) of 48 μg/ml. She was treated with hydrargaphen pessaries 3 mg at night for 28 days. Four months after completion of this regimen trichomonads were not present in the vaginal secretions.

Suppository treatment for trichomoniasis failed in two of five women who developed diarrhoea within one hour of insertion.

FOLLOW UP

Sixty-seven patients attended for follow up four weeks after treatment; of these 12 had trichomoniasis. After consideration of their histories, nine women had been reinfected and three had had a relapse, for which no identifiable cause was found.

The side effects of treatment are shown in table II.

TABLE II *Side effects in 79 patients treated with metronidazole in a single rectal dose*

Side effects	*No of cases (%)*
None	61 (77)
Nausea	5 (6)
Unpleasant taste	4 (5)
Diarrhoea	5 (6)
Abdominal discomfort	3 (4)
Vomiting	1 (1)

Discussion

Metronidazole is well established as the drug of choice in the treatment of trichomoniasis; its use by the rectal route has not been previously reported. It is widely used postoperatively in surgery and gynaecology.[3 4 7] The drug is extremely well absorbed from the rectum, and in two hours attains a trichomonacidal concentration in the plasma, which is maintained for at least 24 hours. The single-dose rectal treatment is completed in the clinic, is cheaper than oral treatment, has a high acceptability rate, and minimises irregular and incomplete treatment (62 patients found that treatment by the rectal route was acceptable). The use of a plastic proctoscope helps in positioning the suppository high in the rectum; this prevents early bowel movement. In this series a cure rate of 94% was achieved. Thus, metronidazole suppositories appear to have a definite place as an alternative to oral treatment. Women with gonorrhoea require rectal examinations in the clinic and they may be treated for trichomoniasis at the same time.

I thank Dr L Cohen for his permission to report on these patients under his care.

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Br J Vener Dis 1982;**58**:259-62

Consulting patterns after a television programme on sexually transmitted diseases*

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SUMMARY The effect on the work load in one of two clinics featured in a television programme devoted to the sexually transmitted diseases was monitored. The patients' source of referral was recorded before and after the programme and the number of telephone inquiries noted during the same periods. For the two weeks after the programme 18% of the male patients and 13% of the female patients attending the clinic came as a result of watching it. A sexually transmitted disease was diagnosed in 30% and 22% of those men and women respectively. This proportion of positive diagnoses was lower than for other sources of referral. The mean number of telephone inquiries rose by 56% after the programme.

Introduction

One of the problems facing those working in the specialty of genitourinary medicine, in which most patients are self-referred, is how best to encourage patients to attend clinics. In particular data on how effective education through the media—by newspaper articles, radio, and television programmes—is in informing the public about the diseases and where to seek treatment are limited and difficult to obtain.[1-5]

In April 1981, BBC television showed a 50-minute programme on the sexually transmitted diseases (STDs). This "Man Alive" programme discussed the increase in the incidence of the diseases and the clinical symptoms and investigation of the more important infections. Two hospitals (Middlesex and Westminster hospitals) and their consultants were featured extensively during the programme. Since I expected that this would have an effect on the work load in these two clinics as well as in others the effect of the programme on this department, and whether this method of presenting information to the public encouraged patients with STDs to attend the clinic, was studied.

Patients and methods

Records were kept of the patients' sources of referral to the clinic for two weeks before and after the programme and of any telephone inquiries during the same periods. The referral source was recorded only for new patients who had never attended the clinic previously. The consulting doctor was asked to note on a special recording sheet the exact way in which the patient had heard of the clinic and been referred. The following options were listed: (a) letter (general practitioner, family planning/pregnancy advice clinic, and other); (b) verbal (sexual contact, friend, and family planning/pregnancy advice); (c) contact slip produced by the patient; (d) educational/advertising (pamphlets, toilet notices, television programmes, radio programmes, newspapers, or magazine articles); and (e) other reasons.

For administrative reasons the monitoring of telephone inquiries was limited to female patients. All calls were answered by the nursing staff, who were asked to record details of their conversation in a standard manner. Since it was not always possible to distinguish old from new patients, inquiries by both were included.

Results

SOURCE OF REFERRAL

During the two weeks before the television programme a total of 351 new patients were seen in the clinic (190 men and 161 women). In the two weeks after the programme 441 new patients attended (250 men and 191 women). The sources of referral for male and female patients for the two weeks before and after the programme are shown in table I.

Before the programme

The commonest source of referral for both male and female patients before the programme was by verbal

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*A shortened version of this paper has already been published (*Br Med J* 1981:**283**:1395).

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Accepted for publication 2 February 1982

TABLE I *Source of referral to clinic before and after television programme*

Source of referral	Men (%) Before (n = 190)		Men (%) After (n = 250)		Women (%) Before (n = 161)		Women (%) After (n = 191)	
Letter	8·4		11·6		6·2		12·6	
Verbal								
Sexual contact	6·3		4·8		9·9		9·4	
Friend	38·4		28·4		20·5		17·3	
Family planning/pregnancy advice clinic		54·7		39·2	36·0	68·9	20·4	52·3
General practitioner	9·0		5·6		2·5		5·2	
Other	1·0		0·4					
Contact slip	3·7		2·4		13·1		7·8	
Television programme(s)	0·5		18·0				12·6	
Toilet notices	1·6		0·8					
Radio/newspaper/magazines	5·3		2·4				1·6	
Telephone book/gay switchboard	15·3		8·8		5·0		4·2	
Other	10·5		16·8		6·8		8·9	
Total	100·0		100·0		100·0		100·0	

contact: with a sexual contact, a friend, a family planning or pregnancy advice clinic, or a general practitioner. For male patients the commonest source was a friend who was not a sexual contact, whereas for women this was the second commonest source after family planning and pregnancy advice clinics. Apart from these clinics, the medical and ancillary professions played little part in referring patients to the clinic. Eight per cent of men and 6% of women were referred with a doctor's letter, either from their general practitioner or a family planning clinic. The telephone directory and "gay" switchboard produced more male consultations (15%) than visits to and referrals by general practitioners. The media were rarely cited as the source of referral by men (television programmes 0·5%, radio, magazine, and newspaper articles 5%) and never by women.

Some of the "other" sources of referral provided an interesting insight into some rarer methods: for example, a prostitute told her client to go to the clinic with his penile wart, a wife found her husband's appointment card, and a patient asked her taxi driver to take her to the "best" clinic.

After the programme

After the television programme the commonest source of referral for men was still by verbal contact. Nevertheless, 18% of male patients visited the clinic as a direct result of watching the television programme. Similarly, the commonest source of referral for female patients was still by verbal contact, but 13% of patients attending during the two weeks after the programme indicated that it had been the reason for their attendance.

DIAGNOSES

The eventual diagnoses made in patients attending after the programme were recorded in relation to the source of referral (table II). The 250 male patients were responsible for 256 diagnoses or cases, since some had more than one disease at the time of consultation. Fifty per cent (129) of male cases seen during the two weeks were sexually transmissible in origin (non-specific genital infection 64, gonorrhoea 21, genital warts 14, genital herpes 14, syphilis 6, pediculosis pubis 4, trichomoniasis 4, and scabies 2).

TABLE II *Diagnosis by source of referral to clinic after television programme*

	Men						Women						
	Total No		Diagnosis				Total No		Diagnosis				
Source of referral	Cases	Patients	STD (%)	Other diseases (%)	NAD (%)	Total (%)	Cases	Patients	STD (%)	Candidosis (%)	Other diseases (%)	NAD (%)	Total (%)
Letters	31	29	64·5	25·8	9·7	100·0	25	24	36·0	16·0	16·0	32·0	100·0
Verbal	99	98	57·6	12·1	30·3	100·0	108	100	32·4	15·7	13·0	38·9	100·0
Other	80	78	47·5	20·0	32·5	100·0	47	43	42·6	25·5	4·2	27·7	100·0
Total of sources excluding television programme	210	205	54·8	17·1	28·1	100·0	180	167	35·6	18·3	11·1	35·0	100·0
Television programme	46	45	30·4	23·9	45·7	100·0	27	24	22·2	29·6	14·8	33·4	100·0
Total of all sources	256	250	50·4	18·3	31·3		207	191	33·8	19·8	11·6	34·8	

$\chi_3^2 = 9{\cdot}096$, $P < 0{\cdot}02$

The next most common diagnosis (31% of cases) was "nothing abnormal detected". The remaining cases (18%) were non-sexually transmitted conditions that required advice or treatment.

The contribution made to the various diagnostic categories by those patients who had watched the programme can be compared with that made by patients from the other referral sources both separately and together. Although 30% of television viewers had an STD, this source of referral produced a lower yield of positive diagnoses than any other (letters 64%, verbal 58%, and "other" 47%). All the sources together excluding television also produced a higher yield than television (55% compared with 30%). The commonest diagnosis among those attending as a result of the programme was that nothing abnormal was found (46%). In contrast, 28% of patients from other referral sources were given this diagnosis. The difference in diagnoses between those coming to the clinic as a result of the programme and those referred by other sources was statistically significant ($P<0\cdot02$).

The 191 new female patients seen in the clinic after the programme were responsible for 207 diagnoses or cases. Most of these were cases in which nothing abnormal was found (35%). In 34% (70 cases) a sexually transmitted disease was diagnosed: non-specific genital infection 21, trichomoniasis 14, gonorrhoea 14, genital warts 13, genital herpes 5, syphilis 2, and pediculosis pubis 1. The remaining cases were either of candidosis (20%) or other diseases that required treatment or advice but were not sexually transmitted (12%).

Like the male patients women attending as a result of the programme produced a lower yield of STDs (22%) than any other source of referral ("other" 43%, letters 36%, and verbal contact 32%). All the sources together excluding television produced a higher yield than television (36% compared with 22%), although this difference was not statistically significant. The two commonest diagnoses among patients attending as a result of the programme were "nothing abnormal found" (33%) and candidosis (30%).

TELEPHONE INQUIRIES

The total number of telephone inquiries made by female patients during the two weeks before the television programme was 169 (mean 16·9 a day) and during the two weeks after 264 (mean 26·4 a day). This represents a rise in calls of 56%, which is statistically significant ($P<0\cdot01$). Not all the telephone inquiries after the programme resulted from it, but it was not possible in all instances to elicit whether the programme had caused the inquiry. The nurses answering the telephone often found it hard to gather this information and had the impression that patients were reluctant to admit to having seen it lest they be considered hypochondriacal. Despite this, the increase in calls was clear-cut and was probably due to the television programme. This is partially confirmed by the fact that the mean number of calls a day dropped to 12·5 in the third and fourth weeks after the programme, when the effect and impact would have worn off.

Discussion

The present study has shown that for two weeks after the television programme just under one-fifth of male patients and one-eighth of female patients attended this clinic as a result of the programme and that an STD was diagnosed in less than one-third of the men and one-quarter of the women. This proportion of positive diagnoses was lower than for other sources of referral. It would be too simple an interpretation to suggest that the programme resulted in only the anxious and hypochondriacal seeking advice since STDs can often be asymptomatic, and in women even the presence of symptoms does not indicate disease. These two problems make it difficult for a person to decide whether or not they have a disease and need to seek advice. Any health information must emphasise these points about symptoms, but unless the information is expanded on a situation will occur in which a large number of patients will be found to have no disease or to have commoner conditions (for example, candidosis) with identical symptoms to those of the real and serious STDs. The information needs to be expanded so that patients can determine to what extent they are at risk. For example, a patient at high risk would be one with a recent change in sexual partner, either of their own or of their partner, multiple sexual contacts, recurrent or persistent symptoms after treatment, and symptoms in sexual partners. Physicians working in genitourinary medicine, who are aware of the poor discriminating power of symptoms and the asymptomatic nature of the diseases, have rightly encouraged patients to attend for routine examinations. This approach should continue but would produce a higher yield of cases of STD if information about the risk of disease was publicised. Even though the work load after the programme was not excessive, future programmes should be more finely tuned to disseminate this information. The media are unlikely to cease to be interested in STDs, but they could benefit from our experience after the television programme. Goodchild[6] makes it clear that the programme producer is more interested in journalism than in providing a public service; "In dealing with the groups at risk and providing them with crucial information, such programmes (on STD) seem to be of only marginal use". This is also suggested by Schofield,[7] who found that young people rarely (6%) admitted that television had been the source of their information about STDs.

An extremely elaborate prospective research project would be needed to assess accurately the true effect of a television programme, media or health education campaign. Samples of non-attenders as well as attenders and of those who had modified their chances of contracting disease as a result of the new information on STDs would need to be studied. Hopefully, those individuals watching the programme, who had no need to attend a clinic, will have received enough information to do so should they develop a disease or see themselves at risk in the future. Even though most patients coming to the clinic as a result of the programme had no disease they will continue to be sexually active and some will contract a disease in the future. Thus, the education afforded by their attendance and a sympathetic reception should both help to prevent disease and, hopefully, encourage those at risk to attend a clinic more readily again at a later date.

I am grateful to all the doctors and nurses working within the clinic for their help in collecting the information and to Elizabeth Belsey for her assistance.

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Br J Vener Dis 1982;**58**:263-8

Social factors in homosexually acquired venereal disease

Comparison between Sweden and Australia

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SUMMARY The prevalence of venereal disease was studied in homosexuals in two countries, Sweden and Australia, which are similar apart from their different legal and social attitudes to homosexuality. Social attitudes were not generally associated with differences in the numbers of infections and reinfections in homosexual men with sexually transmitted diseases. Using a non-clinical sample there was some evidence that sexually transmitted diseases in homosexuals are grossly overestimated if cases rather than individuals are used as an index. Furthermore, the incidence of syphilis was related to the numbers of partners and the latency of symptoms in both societies.

Introduction

It has been recognised for some time that the social context in which sexually transmitted diseases (STDs) occur is important in determining such factors as incidence, prevalence, socioeconomic status of the infected individual, race, and age grouping. Hart[1] goes so far as to comment that "Venereal disease is not primarily a medical problem. It is instead a social one dependent on those factors which influence attitudes and behaviour . . . ." This has been most apparent from cross-cultural studies and from studies which examine the social climate in which the sexual behaviour takes place. For example, Arya and Bennett[2] noted that in East Africa the traditional inverse relationship usually found between social class and the incidence of gonorrhoea was not evident, with venereal disease being a major health problem in universities. This is in clear contrast to western societies, where the incidence of gonorrhoea is much higher in lower socioeconomic groups.[3 4]

With homosexuality and STD the variables of social climate, attitudes at particular clinics, basis of medical statistics, and status of minority groups become increasingly important.[5 6] Social climate was recognised as critical in improving the task of the venereologist as far back as 1971, when Morton[7] commented that from the point of view of contact tracing the English homosexual law reform of 1967 was a most welcome liberalisation of the sex laws. This factor was particularly evident in a Finnish study in 1976 by Suhonen *et al*,[8] who reported an increase in the incidence of primary syphilis from 8% before the decriminalisation of homosexual acts between consenting adults in Finland to 50% in 1974-5 four years later, and a notable decrease since then. That the percentage of homosexually transmitted syphilis rose so startlingly without a notable increase in total figures indicates that the change in the law had no effect on the actual incidence of syphilis but only on the accuracy of reporting the source of the infection. Clearly, the legality of a particular sexual act, and community attitudes towards it, will have an effect on the accuracy of reporting. It is unlikely, however, that it would have an effect on actual incidence, although data so far are unclear. The present study tests this hypothesis in a comparison of venereal disease prevalence in Sweden and Australia.

Although percentages of homosexually acquired STD are high, they are misleading. Of the many studies of the incidence of early syphilis by King,[9] the largest sample was 168 cases,[10] of which 60 were homosexually acquired; these cases were, however, all seen in Greater London. Similarly, in Australia the religious fundamentalists over-reacted to the incidence of homosexually acquired syphilis in 1978 in Melbourne (120 cases, 75% of total infections). When viewed in the context of the population of

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Accepted for publication 25 January 1982

Melbourne (2·5 million), of whom half would be men (1·25 million), 65% over the age of 15 (937 500), and at least 5% homosexual (47 000), this rate of infection would represent only 0·2% of the homosexual population.

The incidence of STD in a particular population must be estimated by looking at non-clinical samples; the incidence in male homosexuals cannot be assessed from those who present to public clinics. In one study of private clinics only 9% were homosexuals.[11]

Figures can also be very misleading if they are incomplete. Many cases of STD are treated by private medical practitioners. Cleere *et al*[12] reported that practitioners may often decide to report on the basis of whether they consider it will help in contact tracing. McKenzie-Pollock[13] estimated that nearly 75% of cases of infectious syphilis in the USA were treated but not reported by private practitioners. In Australia Adams[14] reported that less than 10% of cases of gonorrhoea and syphilis seen in private practice were reported. Clearly, estimates of incidence using public statistics are unreliable and social factors probably influence whether individuals present to a public clinic or to a private practitioner.

Furthermore, the problem of estimating the incidence is compounded by the fact that often each new case is counted as a new patient.[15] Such a method of computation is highly suspect if a promiscuous subgroup is being sampled. Studying a group which was not specifically homosexual, Lundin *et al*[16] examined patients who had had four or more infections in two years. These patients had frequent hospital admissions for non-venereal conditions; 40% of their sexual contacts were also repeaters and 92% lived within two city blocks compared with 18% of non-repeaters. Lundin *et al's* study would suggest that if such a trend is also followed in the homosexual population the number of infections is likely to be a very inaccurate estimate of incidence, as repeaters in the sample could account for a high proportion of total infections.

A final problem relating to the incidence of homosexually acquired STD is whether the contact would be described as a homosexual. A study by Humphreys in 1970[17] showed that about 54% of men making sexual contacts in public toilets were married heterosexuals seeking sexual intercourse which was less impersonal than masturbation. Even with bisexual individuals Jeferiss[18] noted that it was less likely that the contact could be named than with homosexuals. Thus a further element of confusion is added.

In the present study we investigated the incidence in a non-clinical homosexual sample to test three main hypotheses: firstly, in two comparable western societies, which differed in legal and social attitude to male homosexuality, there would be significant differences in the number of individuals who acquired STD; secondly, that there would be a cross-cultural difference in the number of individuals who acquired gonorrhoea and syphilis and that this would be related to promiscuity; and thirdly, that there would be a significant number of reinfections among the individuals and that this would vary with the society in which the individual lived. A number of associated hypotheses relating to clinic attendance were also examined in a cross-cultural context.

Subjects and methods

The two societies chosen, Sweden and Australia, are roughly the same size in terms of population (Sweden eight million, Australia 12 million) and have comparable standards of living and levels of technological development and industrialisation. In terms of political structures, Banks and Gregg[19] found that on the two most important political dimensions Sweden and Australia had practically identical factor loadings (0·918 *v* 0·917). More recently, Sidanius *et al*[20] compared sociopolitical attitudes between Sweden and Australia and found the similarity, expressed as a correlation of factor loadings across factors, was as high as 0·972. The major difference between Sweden and Australia is in their attitude to homosexuality; in Sweden there is no legal condemnation and a high degree of official acceptance whereas in the two states from which the sample was drawn in Australia, Victoria and Queensland, the reverse is the case.

SAMPLE POPULATION

The sample consisted of 176 Swedish and 163 Australian male homosexuals. Both samples were collected by giving questionnaires, complete with stamped addressed envelopes with the researcher's name and university department as the return address, to homosexual rights and social clubs in Stockholm and in Melbourne and Brisbane. In all cases, the club was the main homosexual or social rights organisation in the city, and the aims and functions of the three clubs selected appeared to be almost identical. The response rate was 44% for Stockholm and 46·6% for Melbourne and Brisbane. This rate was based on the number of questionnaires given to the clubs compared with the number of men on their mailing lists. Some differences were apparent between the sample populations (all tests were one-tailed unless otherwise stated). Significantly more Australians than Swedes ($P<0·01$) had religious beliefs. A large proportion of the congregation at an American church, the Metropolitan Community Church, is predominantly homosexual. No such equivalent exists in Sweden. Significant differences also existed in the social status of parents; Australians reported a slightly higher proportion of upper-class parents. Again, this is probably due to the lack of distinct class differences in Swedish society, where most of the population would consider themselves middle-class. Had there been genuine and significant class differences, these would have been reflected in differences in educational background.

The only other significant difference between the samples was in the time elapsing since the respondents became homosexually active (Australia, mean 12·7 years, SD 12·1; Sweden, mean 10·1 years, SD 7·4; t = 2·10, P<0·03). This difference occurred possibly because in Sweden social centres exist for individuals when they first define themselves as homosexual probably more so than in Australia. Nevertheless, it is difficult to see how any of these differences might systematically affect the results.

In general, there was a high degree of similarity between samples on important variables. Age range did not differ significantly (Australia, mean 32·0, SD 11·4; Sweden, mean 30·9, SD 7·4; t = 1·04, NS) nor did years of education (Australia, mean 13·3, SD 3·6; Sweden, mean 12·7, SD 5·3; t = 1·28, NS). The age at which individuals first realised that they were homosexual did not differ significantly between samples (Australia, mean 12·3, SD 11·9; Sweden, mean 14·6, SD 5·8; t = 1·78, two-tail, NS). Thus, there were no substantive differences between the Swedish and Australian samples. Given the large sample studied, the lack of significant differences suggests that the two samples were reasonably well matched.

QUESTIONNAIRE

The questions asked were part of an anonymous wider questionnaire comparing homosexual men in Sweden and Australia. Respondents were asked whether they had had gonorrhoea, syphilis, or any other venereal disease (able to be specified) and the number of times they had had each. Further questions asked whether the respondent had visited a public clinic or local medical practitioner on the last occasion they contracted a venereal disease, whether they admitted the source of infection was homosexual, whether they had had any adverse experiences in a public clinic, whether they went to a particular clinic in preference to any other, and whether they knew the individual by whom they had been infected on a total of five possible occasions and whether they knew any details which would make it possible to contact the individual for a total of five possible occasions for each question. Responses were either yes or no. Finally, individuals were asked the average number of different partners they had had each month over the past year. Ten questionnaires from each sample in which respondents identified themselves were subsequently compared with interviews with the individuals as a check on accuracy; no discrepancies were found. Swedish questionnaires were translated into Swedish by two individuals and checked for accuracy by a third.

DATA ANALYSIS

Analysis was by the χ^2 test for the categorical data and by Student's t test for the data which had interval or ratio scales. χ^2 results were calculated on absolute numbers in all cases, but in some tables raw percentages are also given to enable clearer comparisons between countries.

Results

The results are given in tables I-IV.

TABLE I *Incidence of gonorrhoea and syphilis and mean number of infections in two homosexual sample populations*

	Australia	*Sweden*
Gonorrhoea*:		
Ever had	47	68
Never had	110	108
Syphilis†:		
Ever had	13	23
Never had	144	153
Mean No of infections:		
Gonorrhoea‡	1·904 (SD 0·30)	1·845 (SD 0·36)
Syphilis§	2·614 (SD 0·34)	2·394 (SD 0·23)

SD = standard deviation; NS = not significant

* χ^2_1 = 4·156, P<0·05

† χ^2_1 = 1·516, NS

‡ t = 1·51, P<0·05 (one-tailed)

§ t = 0·54, NS

TABLE II *Percentage of reinfections occurring in two homosexual sample populations*

	No of occasions				
Reinfections (%)	*1*	*2*	*3*	*3-6*	*7-9*
Gonorrhoea*:					
Australia (n = 47)	38·6	27·3	15·9		18·2
Sweden (n = 68)	39·4	30·3	12·1		18·2
Syphilis†:					
Australia (n = 13)	68·8	25·0		6·2	
Sweden (n = 23)	81·8	4·5		13·6	

* χ^2_4 = 0·72, not significant

† χ^2_4 = 19·41, P<0·01

TABLE III *Percentage of two homosexual sample populations with gonorrhoea and syphilis in relation to number of sexual partners*

No of partners	*% Individuals* *Infected*	*Never infected*	*n*
Syphilis:			
High (>2/month)	24·7	75·3	30
Low (<2/month)	20·2	79·8	28
		$\chi^2_1 = 2{\cdot}229$, NS	
High (>4/month)	20·5	79·5	20
Low (<4/month)	10·5	89·1	38
		$\chi^2_1 = 4{\cdot}289$, $P<0{\cdot}01$	
Gonorrhoea:			
High (>2/month)	45·7	54·3	163
Low (<2/month)	30·7	69·3	155
		$\chi^2_1 = 6{\cdot}543$, $P<0{\cdot}01$	
High (>4/month)	45·8	54·2	83
Low (<4/month)	32·8	67·2	235
		$\chi^2_1 = 3{\cdot}956$, $P<0{\cdot}05$	

TABLE IV *Percentage of infections and reinfections in two homosexual sample populations*

	% Total No ever infected (n)	*% Infections which were reinfections (n)*
Australia	49·7 (64)	54·9 (78)
Sweden	47·7 (87)	49·2 (81)
	$\chi^2_1 = 1{\cdot}29$, NS	

NS = not significant

Discussion

The similarity of incidence in STDs between Sweden and Australia appeared to confirm social differences for gonorrhoea but not for syphilis (table I). There was no significant difference between the two countries in the proportion of respondents who had ever had syphilis; however, a higher proportion of Swedes had had gonorrhoea than Australians. It is interesting that in the non-homosexual population gonococcal infections tended to outnumber treponemal ones, and the trend for Sweden was closer to this than that for Australia. This would suggest that, where homosexuality is better accepted and regarded as less abnormal, the pattern of infections might be more similar to that for heterosexuals. The general trend in the two countries, however, for 1978 was that STDs were more common in men in Stockholm (syphilis 0·21/1000; gonorrhoea 4·23/1000) than in Australia (the figures are for the state of Victoria, of which Melbourne is the capital) (syphilis 0·03/1000 and gonorrhoea 1·01/1000). This may be due to differences in data collection, however, as not all private practitioners in Australia notify cases despite the requirement to do so.[14] The proportion of cases of homosexually acquired syphilis has been estimated at about 75% in Melbourne (Bradford, personal communication) and about 50% in Stockholm (Wallin, personal communication). No estimates are available for gonorrhoea. It is, therefore, possible only to speculate about the pattern of treponemal and gonococcal infections in the two populations. Hart[1] has already suggested that the high prevalence of syphilis, which is usually confined to underprivileged or socially deprived groups, reflects the shortcomings of a particular society rather than the intrinsic nature of disease propagation. With regard to the number of times individuals in the two societies were infected (table II), there is no difference for gonorrhoea but a significant difference for syphilis; Australians appeared to be reinfected with syphilis more often than Swedes. Multiple reinfections were, however, more common in Swedes, which tends to contradict the argument about social acceptance. While the data should be interpreted cautiously they do not suggest that the more open a society's acceptance of homosexuality is, the more closely homosexual STD patterns will approximate those of the non-homosexual group.

It has been suggested that the different trends in incidence of gonorrhoea and syphilis in homosexuals (regardless of social climate) may have been related to the length of the period between infection and appearance of symptoms. For gonorrhoea this ranges from three to five days and for syphilis from nine days to three months with a mean of three weeks. One explanation for the higher incidence of syphilis in homosexuals might be the number of sexual partners. Thus, if an individual averages one new partner a week, gonorrhoea will be noted and treated before contact with the next partner. Syphilis, however, may be passed on to two other partners before symptoms become obvious. Does this account for the higher incidence of syphilis in homosexual men? The data analysis of STD type by number of partners a month showed a significant result for gonorrhoea when the split was made at two partners a month; for syphilis the result was not significant (table III). When the split was made at four partners a month, however, the result for gonorrhoea remained essentially the same but that for syphilis was statistically significant.

These data strongly suggest, therefore, that there is a clear association between homosexuality and syphilis only in terms of frequency of partner change. The individual with the greater number of partners (probably an average of over three or four a month) is likely to infect others before his own infection is recognised. The disproportionate figure for homosexually acquired syphilis would appear, therefore, to be explained by those homosexuals who tend to have a relatively high number of different partners.

There were, however, no significant differences between the mean number of partners a month in the two countries (Sweden, mean 3·8, SD 6·6; Australia, mean 3·7, SD 4·2: $t = 0 \cdot 19$, NS). It cannot, therefore, be argued that any differences between the two countries are the result of frequency of partner change.

It was also suggested that the reported incidence of homosexually acquired STD would be overestimated if each case was counted as a new patient, as in some analyses. Table IV compares the number of infections and reinfections (regardless of type of STD) between Sweden and Australia. While there were no statistically significant differences between the two countries, with the exception of the different reinfection rates for syphilis reported above, it is interesting that the proportion of the total sample ever infected with STD was high, slightly under 50%. This result is surprising, even though the population sampled was a high-risk one recruited in homosexual clubs. It is not, of course, representative of the homosexual population as a whole. Nevertheless, from these data nearly half the individuals who patronise homosexual meeting places have at some time contracted a venereal disease. The hypothesis that only a small proportion of homosexuals actually contract STD must therefore be rejected. Clearly, social climate is not a significant influence on the total numbers of infections and reinfections. That the number of reinfections was a significant proportion of the total is clearly supported by the data (table IV). Almost 55% of cases reported in Australia would appear to be reinfections and nearly 50% in Sweden.

Such a finding has very clear implications both for the computation of STD incidences and for identifying an at-risk subgroup. It also reinforces the point made by Hull[5] that there is often a strong bias in some medical statistics towards homosexually acquired venereal diseases. Emphasis, therefore, should be on a group of individuals particularly prone to reinfection and not on the total homosexual community. In general, these data suggest that those individuals who regularly patronise homosexual meeting places are most at risk and that a small core accounts for over half of the cases. The point made by Lundin *et al*[16] regarding a core of reinfectors appears to apply accurately to homosexuals also.

Social bias regarding attendance either at a public clinic or a private medical practitioner was very clear between the two countries (Australia, 38·7% public clinic; Sweden, 82·0% public clinic: $\chi^2_1 = 33 \cdot 09$, $P < 0 \cdot 001$). The significant difference indicated that a far greater proportion of Swedes attended public clinics rather than private medical practitioners, although this term is perhaps misleading in Sweden where most medical practitioners are employed by the State. It cannot be argued that this was due to adverse experiences in clinics, as there was no significant difference between the two samples in such experiences (Australia, 14·1%; Sweden, 14%: $\chi^2_1 = 0 \cdot 039$, NS). There was a significant difference in preference for a particular clinic or practitioner between Swedish and Australian homosexual men (Australia 48·6%; Sweden 28·2%: $\chi^2_1 = 5 \cdot 576$, $P < 0 \cdot 02$). This suggested that Australians preferred an accepting medical practitioner, because of the stigma and legal censure still attached to homosexuality in Australia. Supporting this, Morton[21] noted reports from Western Australia that most STDs were dealt with in the private sector. This does question, however, the reliability of the figures, since a substantial proportion of private practitioners do not report venereal infections.[12-14] Furthermore, estimates need to be made from a non-clinical population, as in the present study, in order to make general observations. Even so, it is doubtful if the present data can be applied to other than homosexual men who frequent homosexual meeting places.

Because of the antihomosexual attitudes in Australia and the more accepting attitudes in Sweden, a significant difference would be expected between homosexuals in the two countries in the number admitting that the infecting contact was a homosexual one. This was not the case (Australia 78·9% admitted; Sweden 79·5% admitted: $\chi^2_1 = 0 \cdot 009$, NS). This may be explained by the fact that high proportions of Australian homosexuals visited accepting medical practitioners and therefore did not need to hide the source of infection. Thus, the response to greater antihomosexual pressures is probably to find and visit accepting practitioners or clinics. Any social factor present must be related to openness of presentation as a homosexual and directed to the source of treatment rather than to any difference in sexual preference. The social climate relating to homosexuality did not have any direct effect on admission of source of infection.

These data do not show that social factors are associated with major differences in the presentation of homosexual men with STD. This is apparent regarding the incidence of syphilis and the proportion of reinfections but not the place of presentation. In particular the composition of health statistics for the STDs can be questioned. The number of cases of homosexually acquired venereal disease is grossly overestimated if cases rather than patients are recorded and probably grossly underestimated where there are antihomosexual attitudes which militate against the individual attending a public clinic where figures are likely to be reported. Any approach to venereal disease education and to epidemiology must take such factors into account. The evidence does not appear to support the hypothesis that social attitudes to homosexuality have any pronounced effect on infection or reinfection rates.

The research on which this paper is based was funded by a travel grant from the Faculty of Arts of the University of Melbourne. The author would like to thank the members of RFSL (Stockholm), CAMP (Brisbane), and Society Five and MCC (Melbourne) for their assistance in providing data for this study.

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Br J Vener Dis 1982; **58**: 269-70

Guillain-Barré syndrome associated with hepatitis A in a male homosexual

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SUMMARY A 48-year-old male homosexual developed the Guillain-Barré syndrome in association with acute hepatitis. The hepatitis A virus was almost certainly transmitted sexually. Since the incidence of viral hepatitis is high in active male homosexuals, they are particularly at risk of developing such complications.

Introduction

Sexually transmitted viral hepatitis is an increasingly important problem in active male homosexuals.[1 2] Although hepatitis B infection usually causes a more severe illness than hepatitis A and may cause chronic liver disease, hepatitis A is more common[1] and may occasionally cause other serious complications as well as fulminant hepatic failure. We report the potentially fatal complication of Guillain-Barré syndrome with serologically confirmed hepatitis A in an active male homosexual.

Case report

A previously healthy 48-year-old man felt unwell with headache, fatigue, and myalgia. Three days later he noticed dark urine and paraesthesia in his hands and feet. The next day his legs and upper arms were weak and he became jaundiced.

He had not had any injections, tattoos, or blood transfusions and did not abuse drugs. He had not travelled abroad recently nor had any contact with jaundiced people. Nevertheless, he had been an active homosexual for 10 years. He had between 20 and 25 new partners each month, with whom he had oroanal contact.

CLINICAL FINDINGS

On admission to hospital four days after the onset of his symptoms he was slightly jaundiced, but there were no signs of chronic liver disease. There were, however, pronounced neurological abnormalities. He was hypotonic and areflexic. Power was generally reduced in the arms and legs, particularly proximally. Joint-position sense and vibration sense were diminished in the feet, but sensory testing was otherwise normal.

LABORATORY FINDINGS

The serum liver function tests were abnormal. Serum aspartate aminotransferase was 237 IU/l (normal 5-40 IU/l) and alkaline phosphatase 458 IU/l (normal 30-110 IU/l); serum bilirubin concentration was 29 μmol/l (normal 5-17 μmol/l). Hepatitis B surface antigen (HBsAg) and antibody (HBsAb), hepatitis B core antigen (HBcAg), and IgM antibody were all absent; serological test results for recent Epstein-Barr virus and cytomegalovirus infections were negative. Hepatitis A IgM antibody measured by radioimmunoassay was detectable.

COURSE OF ILLNESS

The weakness of the limbs progressed, and three days after admission the patient was unable to walk or feed himself. The following day he developed bilateral lower motor neurone VII cranial nerve palsies and was unable to sit up. He also became dysarthric, but swallowing and the gag reflex remained normal.

A lumbar puncture showed normal pressure (100 mm Hg); the cerebrospinal fluid contained no cells, 0·3 g/l protein, and 3·5 mmol/l (63 mg/100 ml) glucose. In view of these somewhat surprising results nerve conduction studies were performed. Motor conduction in the right median nerve was reduced at 37 m/s and the terminal latency was prolonged (6·8 ms). The F wave latency with elbow stimulation was increased (37 ms). No sensory nerve action potential was detectable. These abnormalities of conduction are consistent with the clinical diagnosis of the Guillain-Barré syndrome.

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Accepted for publication 10 January 1982

The patient made a rapid recovery from hepatitis. By the eleventh day after admission the prothrombin time had improved sufficiently for a liver biopsy to be performed safely; it showed the changes of recent acute hepatitis. Meanwhile, the patient was also recovering power in the limbs and he could walk with a frame 12 days after admission. He was well enough to be discharged three days later. When he was next seen two months afterwards, power in the limbs was normal, but the VII cranial nerve palsies persisted. Five months after the initial symptoms there was further improvement, but a mild left VII cranial nerve palsy remained.

Comment

The presence of IgM antibody against hepatitis A virus identified it as the cause of the acute hepatitis in this patient. Hepatitis A is now considered to be one of the enteric infections that are transmitted sexually among homosexual men. Our patient, who was frequently the oral partner in oroanal contact, was particularly at risk, since Corey and Holmes showed that the acquisition of hepatitis A infection was correlated with this form of sexual activity.[2]

The progressive symmetrical polyradiculopathy, which developed in this patient in association with the acute hepatitis, is typical of the Guillain-Barré syndrome. The diagnosis is essentially clinical and supported by nerve conduction studies.[3] Although the protein content of the CSF is usually high, it may be normal, especially in the early stages, and in some cases remains so.[4]

The major danger in the Guillain-Barré syndrome is paralysis of the respiratory muscles, but the prognosis is generally good. Approximately 75% of patients recover totally,[5] and the delayed improvement of the facial nerve palsies in this patient is typical.

The Guillain-Barré syndrome is often associated with some form of bacterial or viral infection. Respiratory infections are the most common.[6] The association with hepatitis is unusual. In a series of 1100 cases of Guillain-Barré syndrome Leneman[6] reported 11 associated with acute hepatitis. This report[6] and others[7 8] preceded the development of specific serological tests to identify the hepatitis viruses. Subsequently, a few cases of Guillain-Barré syndrome associated with hepatitis B have been reported,[9-11] but there has only been one previous report associated with hepatitis A.[12]

Because the incidence of viral hepatitis is high in active male homosexuals, they may be particularly at risk of developing such complications.

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Br J Vener Dis 1982;**58**:271-2

Strongyloidiasis: is it transmitted from husband to wife?

DAVID I GROVE
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SUMMARY The possible transmission of *Strongyloides stercoralis* from men who had been infected for many years to their wives was investigated. None of the 24 wives was infected, as indicated by the absence of anti-*Strongyloides* antibodies in their serum. Thus, the risk of transmission from husband to wife seems small, although the likelihood among homosexuals is greater.

Introduction

The sexual transmission of enteric parasites has attracted continuing attention since Mylius and ten Seldam[1] in 1962 described a married couple in whom both partners had contracted genital amoebiasis. Protozoal infections are more common than helminth infections, and transmission occurs more frequently, but not exclusively, in homosexual patients.[2-4]

The purpose of this investigation was to determine whether the human helminthic parasite, *Strongyloides stercoralis,* could be transmitted from husband to wife.

Patients and methods

Twenty-four couples, of whom the wife did not live in an endemic area but the husband had been infected continuously for at least 35 years since being a prisoner-of-war in South-east Asia, were studied. The parasitological and clinical features of the men have been described earlier.[5] The couples had been married for many years (range 22-45, mean 35, median 35 years).

Sera were obtained from all 24 couples. The diagnosis of strongyloidiasis was made by a sensitive and specific immunofluorescent antibody assay, in which an anti-*Strongyloides* antibody titre of >1/4 was indicative of infection.[6]

Results

All 24 men had serum antibodies with titres ranging from 1/8 to 1/512. No antibodies were detected in 23 wives while one had a titre of 1/2.

Discussion

Many of the men with strongyloidiasis were concerned to know whether the infection could have been communicated to members of their families, particularly their wives. There have been suggestions that various helminths may be sexually transmitted; these include *Enterobius vermicularis,*[7] *Hymenolepis nana,*[2] and *S stercoralis.*[8]

S stercoralis, however, is a most unusual helminth as it has the capacity to replicate within the same host. This is achieved by the maturation of some non-infective rhabditiform larvae into infective filariform larvae within the intestinal contents. These larvae then either penetrate the intestinal mucosa (internal autoinfection) or the perianal skin (external autoinfection). Consequently, this worm resembles other classes of micro-organisms, such as bacteria or protozoa, in its potential ability to infect other individuals directly without the participation of an intermediate host or the need for a period of development in the external environment.

There are several theoretical means by which infection can be transmitted. A person could be infected by contact with larvae in faeces which had soiled the perianal skin of the partner. Infection could also be acquired during handling and washing of clothes which had been contaminated with faeces. Despite the probability of numerous opportunities for such exposure, none of the women in this study had become infected.

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Accepted for publication 3 March 1982

Thus there seems to be little risk of transmission of strongyloidiasis in normal family surroundings. In view of the life cycle of this parasite, however, the likelihood of transmission among homosexuals would probably be much greater, particularly if the passive partner is infected. Further studies are needed to clarify this point.

The technical assistance of Miss C Maizey is gratefully acknowledged.

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Br J Vener Dis 1982;58:273-4

IUVDT—South-east Asian and Western Pacific regional conference

Report of the Second Regional Conference of the South-east Asian and Western Pacific Branch of the IUVDT held in Christchurch, New Zealand, 23-26 October 1981

The Second Regional Conference of the South-east Asian and Western Pacific Branch of the International Union against the Venereal Diseases and Treponematoses (IUVDT) took place in Christchurch, New Zealand, from 23 to 26 October 1981. Originally it had been planned to be held in Australia but for a variety of reasons this was not possible. As a result Dr W M Platts and his colleagues in Christchurch, who are interested in the sexually transmitted diseases, agreed to arrange the meeting in New Zealand.

The conference organising committee was headed by its co-ordinator, Dr M A Brieseman, who was ably supported by the secretary, Mrs S Wilson. The South-east Asian and Western Pacific Branch of the IUVDT was represented by its chairman, Dr P Vejjabul (Thailand), the vice-chairman, Dr C N Sowmini (Madras, India), the executive secretary, Dr V S Rajan (Singapore), and members of the branch committee. The IUVDT was represented by its president, Dr R D Catterall (London).

Eighty-two delegates took part in the meetings, the strongest contingents being from New Zealand, Australia, and Indonesia. In addition, there were representatives from India, Thailand, Singapore, the United States, and the United Kingdom. The scientific sessions were held in the large lecture theatre of the clinical school of Christchurch Hospital, and the delegates were welcomed by Mr H G Hay, the mayor of Christchurch, Professor F T Shannon, the acting dean, Dr W M Platts, the president of the New Zealand Venereology Society, and Dr V S Rajan (Singapore) for Dr P Vejjabul, the chairman of the Branch. Dr R D Catterall, the president of the IUVDT, gave a brief outline of the history and aims of the Union, and the conference was officially opened by the Honourable G Gair, the minister of health of New Zealand. Dr R C Begg, deputy director of the Division of Public Health, proposed a vote of thanks to the minister.

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Accepted for publication 19 January 1982

Penicillinase-producing gonococci

On the first day there was a review of the global problem of the sexually transmitted diseases by the president, Dr R D Catterall, and an analysis of the situation of antiobiotic resistance, with special reference to penicillinase-producing *Neisseria gonorrhoeae* (PPNG) by Dr P Wiesner (Center for Disease Control, Atlanta). Since 1979 there had been a dramatic 241% increase in reported infections due to PPNG in the United States. Recently, PPNG isolates had shown increasing resistance to tetracycline, cefoxitin, sulphonamides, and trimethoprim. Most strains were of Far Eastern origin but were now endemic in the USA. In an attempt to prevent the spread of these strains a strong case was made for testing all gonococcal isolates for penicillinase production.

Cases of infection with penicillinase-producing *N gonorrhoeae* were reported from Indonesia, particularly from Jakarta and Surabaya. Alternative drugs to penicillin, such as spectinomycin, were too expensive, and cefuroxime and cefoxitin were not yet available in Indonesia. In a small number of patients, however, good results had been obtained with a single intramuscular injection of 2 g kanamycin, a single oral dose of 300 mg rosoxacin, or 3 g thiamphenicol.

An evaluation of the in-vitro and in-vivo efficacy of the cephalosporin antibiotics against PPNG was presented by Dr W O Harrison and his colleagues (Naval Regional Medical Center, San Diego, California). In a small series cefoxitin and ceftizoxime followed by cefonicid and cefotaxime gave the best results. Side effects, including pain at the injection sites, made it essential to mix both cefoxitin and cefonicid with lignocaine.

A total of 20 cases of ophthalmia neonatorum caused by PPNG were reported from Middle Road Hospital, Singapore, by Dr T Thirumoorthy. Treatment with kanamycin eye drops had produced satisfactory results in all cases. Dr V S Rajan described the steady increase in the number of penicillinase-producing strains in South-east Asia; in most large centres between 30% and 50% of the strains produced β-lactamase. Many of the second and third generations of cephalosporins had given good results; both cefuroxime and ceftriaxone given in a dose of 500 mg with 1 g probenecid produced excellent cure rates. Treatment with rosoxacin, however, had been disappointing in Singapore.

Dr J W Tapsall (Randwick, Australia) made a plea for standardisation of gonococcal sensitivity testing. The experience of the Australian Gonococcal Surveillance Service from 1979 to 1981 had shown that the variation in results from different laboratories was the result of the widely different techniques used. Since uniformity of method had been achieved in laboratories in Australia with a particular interest in STD both inter-laboratory and intra-laboratory variation had been eliminated. The recommended laboratory methods were described in detail.

The use of cefuroxime in the treatment of patients with infections due to PPNG was described by Dr N Polnikorn. Forty-eight per cent of all the strains isolated at Ramathibodi Hospital, Bangkok, produced β-lactamase. Serum concentrations of cefuroxime were measured in patients receiving 0·75 g intramuscularly 15 minutes after 1 g probenecid orally and in those receiving 1·5 g cefuroxime preceded by probenecid. The results of treatment were notably better with the higher dosage.

A detailed study of the sensitivities of non-PPNG and PPNG strains isolated in Bangkok to penicillin, thiamphenicol, kanamycin, cefoxitin, and spectinomycin was reported by Dr K Panikabutra from the Bangkok Hospital. The current prevalence of PPNG strains in Bangkok was about 43%. The results of the trial showed that spectinomycin, kanamycin, cefoxitin, and thiamphenicol were effective alternatives to penicillin in the treatment of urethritis due to both PPNG and non-PPNG strains. Thus, thiamphenicol might be the treatment of choice for gonorrhoea in developing countries.

Papers by Dr J S W Frew (Auckland, New Zealand) on gonorrhoea and non-gonococcal urethritis in merchant seamen, by Dr J J Judonarso (Doctor Cipto Mangunkusumo General Hospital, Jakarta) on the incidence of the various sexually transmitted diseases in Jakarta, and by Dr B T Lau (Kuala Lumpur, Malaysia) suggesting that there had been an increase in diagnosed cases of syphilis since kanamycin had been widely used in the treatment of gonorrhoea in Malaysia, were followed by a lively discussion. Anxiety was expressed about the rapid increase in PPNG strains throughout the world. It was generally believed that not enough was being done to monitor the incidence of these strains. There did not appear to be a consistent national and regional policy on treatment, contact tracing, and surveillance, nor did the World Health Organisation seem to be giving clear-cut leadership in an attempt to prevent their further spread throughout the world.

Viral diseases

A session was devoted to the sexually transmitted viral diseases and Dr R D Catterall (London) reviewed the growing problem of these infections and their long-term consequences. A brief review of the viral flora of the human genital tract was given by Dr W Hamilton (National Health Institute, Wellington). Dr Morris Gollow (Perth) described the isolation of various serotypes of adenoviruses from the genital tract, the anorectum, and the conjunctiva. The presence of adenovirus was associated with non-specific urethritis, proctitis, and conjunctivitis. Ocular and genital infections in the form of conjunctivitis and non-specific urethritis were the most frequent findings.

Hepatitis B in a combined dermato-venereological clinic was described by Dr H C M Stringer (Otago University, Dunedin) with special reference to patients requesting dermabrasion of tattoos. In India Dr Kantharej had found the highest incidence of HBsAg among homosexual men and prostitutes. Dr Goh (Singapore) had been unable to find a high incidence of gonorrhoea in a series of 100 patients with condylomata acuminata in Singapore.

Syphilis

Control of syphilis in developed and developing countries was discussed by Dr F E Willmott (Auckland). Three major factors in the recent increase of the disease in developed countries were the high incidence in homosexual men, failure to recognise the disease, and the increased responsibility for the treatment and control by private practitioners. There were also three different factors responsible for the high incidence of the disease in developing countries, namely the eradication of yaws, and with it the removal of immunity to syphilis, the sudden and rapid sociocultural changes associated with development, and the lack of adequate medical facilities. The patchy nature of skilled and informed medical care for patients with sexually transmitted diseases in the South-east Asian and Western Pacific region was stressed.

Low positivity rates in routine serological tests for syphilis and tests on the cerebrospinal fluid were reported by Dr F D Pien and his colleagues (Straub Clinic, Honolulu). The continuation of the routine use of screening tests for syphilis should depend on the reactivity rates in the population and the cost-benefits of treatment. Patients with reactive serological tests should be fully investigated and, if necessary, treated.

The need for quality control of serological tests for syphilis was stressed by Dr D J Merry (Adelaide). An improvement in the accuracy of screening tests was shown by all laboratories which took part in a voluntary proficiency programme. Dr C Ross Philpot (Flinders Medical Centre, South Australia) had measured serum erythromycin concentrations in healthy volunteers after ingestion of 250 mg of base equivalent four times daily. Ingestion of food reduced and delayed the maximum serum concentration but the regimen produced serum erythromycin concentrations appropriate for the treatment of superficial infections. Nevertheless, maximum serum concentrations suitable for deeper infections and those caused by less sensitive organisms required a larger dose of at least 500 mg of base equivalent.

Other papers

Contact tracing was discussed by Mr G M Ineson (Wellington) and Ms Jo Johnston (Wellington), who described local problems and the introduction of new techniques. A joint interview system where patients were interviewed by both a male and female interviewer at the same time, an improved record system permitting more detailed evaluation of contact tracing, and the introduction of contact cards or slips had improved the results of contact tracing.

Other papers included an analysis of the use of routine chlamydial cultures by Dr M M Gollow (Perth), a description of four cases of the FitzHugh-Curtis syndrome by Dr J Keane (Hamilton), a rich pictorial presentation of the gynaecological manifestations of sexually transmitted diseases by Dr C N Sowmini (Madras), and a statistical account of the downward trend of gonorrhoea and gonococcal pelvic inflammatory disease in the United States by Dr P J Wiesner (Atlanta).

The polymicrobial aetiology of genital ulceration was stressed by Dr V S Rajan (Singapore) and the value of the routine cultures for cervical cytomegalovirus discussed by Dr F E Willmott (Auckland). Dr J R L Forsyth demonstrated the superiority of permanent stains, using either trichrome or chlorazole black, over direct wet mounts in the detection of protozoa in faeces from homosexual men and pointed out that chlorazol black was very suitable for occasional use, particularly in clinics for sexually transmitted diseases.

Finally, Dr V S Rajan (Singapore) reported that prostitutes who treated themselves with penicillin had a lower incidence of cervical and rectal gonorrhoea but a higher number of PPNG infections than those who took non-penicillin antibiotics prophylactically.

Business and social functions

Meetings of the South-east Asian Branch of the IUVDT, the New Zealand Venereology Society, and the Australia National Venereology Council were held during the conference and were attended by the President of the IUVDT, Dr R D Catterall (London).

There was a lively social programme for the ladies and a cocktail party and a conference dinner for all those attending the meeting. During an afterdinner speech, Dr R D Catterall pointed out that this very successful meeting would never have taken place without the determination of Dr W M Platts, the organising skills of Dr M A Brieseman, and the attention to detail of Mrs S Wilson.

It was announced that the next meeting of the South-east Asian and Western Pacific Branch of the IUVDT would be held in Bangkok, Thailand, from 24 to 26 June 1983.

R D Catterall
(President, IUVDT)

Br J Vener Dis 1982;**58**:275-6

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Inefficacy of cimetidine in condylomata acuminata

Sir,
Condylomata acuminata form one of the most frustrating ailments to manage. Clinical and in-vitro observations suggest that a refractory course of condylomata may be a reflection of an underlying state of cellular immunosuppression.[1] Recently, cimetidine has been shown to possess immunomodulatory properties; its mode of action is probably related to the inactivation of suppressor cells.[2] In two experimental models[3 4] cimetidine has been highly effective in reducing the number of metastatic deposits and in increasing the life-span of tumour-bearing animals. Since cimetidine was not directly cytotoxic to tumour cells in vitro, its activity could be ascribed to the augmentative effect on the immune response.[4] On the basis of these data we studied the efficacy of cimetidine as an adjuvant in treating chronic condylomata acuminata.

Twelve healthy patients aged 19-39 years (11 men and one woman) entered the study. All had multiple venereal warts of 4-60 months' duration, and all had been unsuccessfully treated before. Local treatment consisted of podophyllin 10-20% in collodion once weekly until all lesions had resolved. Cimetidine 400 mg was given four times daily for eight weeks. Two patients refused further treatment after four weeks because of exacerbation during treatment. Ten patients completed their treatment course, of whom eight had persistent condylomata at the end of the trial period and after 1-3 months' follow up. One patient defaulted, but inquiry by telephone indicated that all the lesions had completely resolved. In only one case could a complete cure be claimed. This patient had frenal lesions only. Side effects were not found. Interestingly, three patients had concomitant common warts on the hands or feet, none of which showed any improvement during treatment with cimetidine.

These findings show that cimetidine has a negligible effect on venereal warts. Thus, a randomised prospective study in this field seems unwarranted.

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TO THE EDITOR, *British Journal of Venereal Diseases*

Post-gonococcal cervicitis and post-gonococcal urethritis

Sir,
We were interested to read of the high incidence of post-gonococcal urethritis and cervicitis in a recent article.[1] We rarely see such conditions in this clinic nowadays.

Because of the reported high rate of coincidental infection with chlamydia, we give all patients with gonorrhoea (male and female) a 10-day course of oxytetracycline (500 mg twice daily). This course is the same as we use for non-specific urethritis (NSU) and contacts of non-specific urethritis, and is given after ampicillin 2 g and probenecid 1 g in one immediate oral dose. We have never regretted giving oxytetracycline in these cases. Such a regimen is associated with a low incidence of post-gonococcal syndromes (3%), low treatment failure rates for gonorrhoea (<1%), and therefore less necessity for long-term follow up. One disadvantage may be that an increased number of women develop candidosis but this is usually diagnosed and treated before the final follow-up visit.

This management may not meet strictly academic ideas of "diagnosis before treatment," but in the absence of chlamydial cultures there seems no practical acceptable alternative (compare NSU and NSU contacts). Even if only a small percentage of men and women treated for gonorrhoea avoid chlamydial epididymitis or salpingitis by this means, it is surely worthwhile. In addition, it decreases the likelihood of partners reinfecting each other with chlamydia.

Yours faithfully,

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S R S Laing
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Reference

1. Arya OP, Mallinson H, Pareek SS, Goddard AD. Post-gonococcal cervicitis and post-gonococcal urethritis. *Br J Vener Dis* 1981;**57**:395-9.

TO THE EDITOR, *British Journal of Venereal Diseases*

Aetiology and management of non-specific vaginitis

Sir,
Dr Dattani and his colleagues[1] are to be congratulated in attempting to tackle the complex clinical and microbiological features of the ubiquitous and distressing non-specific vaginitis. They do, however, seem to be basing their assumption, that most cases of *Gardnerella vaginalis* vaginitis are cured spontaneously, on the 10 cases in their treatment control group who regressed without treatment. I would suggest that this is a little premature. Our experience of *G vaginalis* vaginitis in Sheffield over five years (and 5000 patients) suggests that certainly some cases cured themselves, but this is by no means the rule.

Yours faithfully,

M D Talbot
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Reference

1. Dattani IM, Gerken A, Evans BA. Aetiology and management of non-specific vaginitis. *Br J Vener Dis* 1982;**58**:32-5.

TO THE EDITOR, *British Journal of Venereal Diseases*

False-negative reagin test results to non-agglutinating (incomplete) antibodies

Sir,
We wish to report two cases of clinically active syphilis in which the results of the rapid plasma reagin (RPR) test for measuring antibodies to cardiolipin were negative and the *Treponema pallidum* indirect haemagglutination assay (TPHA) and fluorescent treponemal antibody (FTA) tests were positive. The FTA test result was

positive for both IgG and IgM antibodies. In view of the fact that the IgG antibodies were strongly positive we considered it unlikely that these were cases of early syphilis with low RPR values due to early stage disease.[1] We suspected that these individuals may have produced antibodies of the non-agglutinating or incomplete type and therefore performed a Kolmer complement-fixation test (CFT) using Difco cardiolipin-lecithin antigen (Detroit, Michigan, USA) as well as a Coombs antiglobulin test on both specimens.

The Coombs antiglobulin test was performed using equal volumes of undiluted patients' sera and charcoal particles on to which cardiolipin was adsorbed (VDRL carbon antigen, Wellcome Research Laboratories, Beckenham, Kent). Normal control sera as well as phosphate-buffered saline (PBS 0·15 mol/l) were used as negative controls. The mixtures were rotated for 10 minutes followed by three washing steps in PBS to remove unbound serum components. After the final wash 0·2 ml Coombs reagent (goat anti-human globulin, Institute Pasteur, Paris) was added to the charcoal particles, incubated overnight at 37°C, and assessed the following day for agglutination. The serological results are shown in the table.

The positive results for IgM show that in case 1 the patient had active syphilis. Although the RPR test result was negative the Kolmer CFT and Coombs test, which measured non-specific antibodies to cardiolipin, gave positive results showing the presence of incomplete antibodies to cardiolipin. The results obtained on the second patient (case 2) were similar except that the Kolmer CFT result was negative. This may have been due to the presence of an incomplete antibody with poor complement-binding activity. Darkfield examination for detection of *Treponema pallidum* was not performed.

Clinical diagnoses of active syphilis had been made for both patients, but it was possible only to obtain a case history as well as follow-up sera from the first patient. This 46-year-old man attended the clinic for sexually transmitted diseases on 12 November 1981 complaining of a penile ulcer, which had been present for two weeks. He later also admitted having a urethral discharge for three weeks and denied extramarital sexual contact. On examination the right inguinal lymph glands were greatly enlarged, he had a profuse, thick, grey urethral discharge, and there was a large ulcer at the corona (about 15 mm in diameter). Clinically the ulcer looked like a chancre, but the inguinal lymphadenitis made the diagnosis of chancroid more probable. Smears of the urethral discharge gave negative results for *Neisseria gonorrhoeae* (no culture was performed).

TABLE *Serological test results of two patients with clinically active syphilis*

	Results of serological tests					
	RPR	*TPHA*	*FTA-ABS*			
Patients			*IgG*	*IgM*	*Kolmer CFT*	*Coombs test*
Case 1						
Initial results	–	+	3+	1+	1/64	+
On 4.12.81	–	+	1+	–	–	–
Case 2						
Initial results	–	+	3+	1+	–	+
Wife*	1/16	+	4+	–		

+ Positive; – negative
*Of the patient in case 1
RPR = rapid plasma reagin (test); TPHA = *Treponema pallidum* haemagglutination assay; FTA-ABS = fluorescent treponemal antibody-absorption (test); CFT = complement-fixation test

Treatment consisted of a single intramuscular injection of benzylpenicillin (5 million units); weekly injections of benzathine penicillin (2·4 million units for two weeks; and long-acting sulphonamides (one tablet daily for two weeks). On 4 December all that remained was a small scab at the site of the penile ulcer; the results of serological investigations are shown in the table. The patient's wife also had clinically active syphilis (table).

These results indicate that incomplete antibodies to cardiolipin may occasionally complicate the serodiagnosis of syphilis. The incidence of such antibodies is not yet known but is probably low. The importance of using the TPHA as a screening test with subsequent investigation of positive specimens for IgM antibodies by the FTA-ABS test is emphasised.

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Notices

Medical mycology

A postgraduate course in medical mycology (dermatomycology) is to be held from 16-18 September 1982 at the University of California, San Francisco, USA. For further information please write to: Extended Programs in Medical Education, Room 569-U, School of Medicine, University of California, San Francisco, California 94143, USA (telephone No: 666-4251).

Retirement symposium

A one-day symposium on sexually transmitted diseases is being held in honour of Dr C S Ratnatunga, FRCP(Ed), on Friday 24 September 1982 at the Royal Free Hospital, Pond Street, London NW3. You are invited to contact the Marlborough Department of Venereology at this address for details.

Br J Vener Dis 1982; **58**: 277-80

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Diagnosis of syphilis

A LUGER (Vienna University, Austria). *Bull WHO* 1981;**59**:647-55.

General paralysis of the insane

I FRASER (Royal Victoria Hospital, Belfast, UK). *Br Med J* 1981;**283**:1631-2.

A survey of 251 patients with acute syphilis treated in the collaborative penicillin survey of 1943-1950

RH KAMPMEIER, A SWEENEY, RW QUINN, *ET AL* (Center for Disease Control, Atlanta, GA, USA) *Sex Transm Dis* 1981;**8**:266-79.

The profile of neurosyphilis in Denmark. A clinical and serological study of all patients in Denmark with neurosyphilis disclosed in the years 1971-1979 inclusive by Wassermann reaction (CWRM) in the cerebrospinal fluid

A PERDRUP, BB JØRGENSEN, AND NS PEDERSEN (Hvidovre Hospital, University of Copenhagen, Hvidovre, Denmark). *Acta Derm Venereol* 1981 suppl **96**:3-14.

Using the Syphilis Index at the State Serum Institute, Copenhagen, which contains national serological data from blood and cerebrospinal fluid (CSF) specimens since 1929, this series of 43 men and 12 women with neurosyphilis probably comprises all known cases encountered in Denmark during the nine-year period. Classified according to clinical features there were two cases of early syphilitic meningitis, 17 of asymptomatic neurosyphilis, 19 of meningovascular syphilis (six with inflammatory reactions of brain base, three of the convexity of the brain, and three of the spinal cord, and seven with syphilitic arteritis), one of gumma, and 16 of parenchymatous neurosyphilis (12 of general paralysis of the insane, three of tabes dorsalis, and one of optic atrophy). A wide range of alternative diagnoses had been made, which indicated a low index of suspicion among physicians.

All but 17 patients had a CSF white cell count exceeding 5×10^6/l cells and only 17 had a CSF protein content less than 500 mg/l, both being normal in only nine. The TPI reaction was strongly reactive in the blood of all patients and reactive in the CSF of 51 patients.

The penicillin dosages (12·2-21 million units) were those currently given and the treatment was considered beneficial in most cases. Because of the increasing rarity of all types of tertiary syphilis there has been little doubt about the efficacy of current treatment of early syphilis. There was no evidence that any cases of neurosyphilis in this series arose from the failure of antibiotic treatment of early syphilis.

R R Willcox

(Reprinted from *Abstracts on Hygiene* by permission of the Editor).

Rectal mass caused by *Treponema pallidum*: confirmation by immunofluorescent staining

TC QUINN, SA LUKEHART, S GOODELL, *ET AL* (United States Public Health Service Hospital, Baltimore, MD, USA) *Gastroenterology* 1982;**82**:135-9.

Anorectal syphilis is infrequently diagnosed because it is often asymptomatic, and clinicians may fail to examine routinely the anal canal or rectum for chancres in homosexual men. Even when the patients are symptomatic the lesions can be misdiagnosed as traumatic lesions, fissures, non-specific proctitis, or polyps.

A case is reported of a 37-year-old homosexual man with mild anorectal symptoms, a diffuse macular papular rash with constitutional symptoms (erythrocyte sedimentation rate 73 mm in one hour and a reactive serological test for syphilis). Sigmoidoscopy showed a 2 × 2 cm indurated rectal mass 5 cm above the anal verge, which was darkfield-positive for *T pallidum*, and biopsy showed diffuse infiltration of plasma cells and lymphocytes. *T pallidum* was present on indirect immunofluorescence and the lesion resolved after treatment with penicillin.

R R Willcox

(Reprinted from *Abstracts on Hygiene* by permission of the Editor).

Cerebrospinal fluid penicillin levels during therapy for latent syphilis

J DUCAS AND HG ROBSON (Department of Microbiology and Immunology, Montreal, Quebec, Canada). *JAMA* 1981;**246**:2583-4.

Thirty-three patients with latent syphilis (diagnosed by positive serological results to VDRL and FTA-ABS tests) were studied to determine the cerebrospinal fluid (CSF) absorption of penicillin G benzathine. They were given either 2·4 or 4·8 megaunits of penicillin G benzathine im weekly for three weeks with or without probenecid 500 mg orally four times daily, thus establishing four groups Serum concentrations of penicillin were monitored weekly, starting seven days after the first injection. The stated objective was to achieve penicillin concentrations in both the blood and CSF of at least 0·03 units/ml, previously defined as the concentration required to cure early syphilis.

The mean serum concentration one week after the third penicillin injection was 0·32 units/ml using 2·4 megaunits increasing to 0·41 units/ml with probenecid. With 4·8 megaunits the concentration rose to 0·75 units/ml. Only two out of six patients given the 4·8 megaunit regimen with probenecid had CSF penicillin concentrations greater than 0·03 units/ml. Because of this the authors recommend the use of intravenous penicillin G sodium for neurosyphilis as first-line treatment rather than benzathine penicillin.

In an accompanying editorial Rein points out the lack of information on the use of penicillin G benzathine in neurosyphilis with cure rates, in two small series, ranging from 67-81%. Most available information is purely anecdotal. The results in the above paper are limited as only patients with latent syphilis were studied. In cases with meningeal inflammation higher CSF concentrations of penicillin would be expected, but even in active neurosyphilis CSF concentrations lower than 0·03 mg/ml have been reported with penicillin G benzathine. Probenecid increases the CSF concentration in several ways but may actually interfere with the accumulation of penicillin in brain tissue; therefore raising the dose of penicillin may be more reliable. In view of these doubts high-dose intravenous crystalline penicillin G is recommended for neurosyphilis on theoretical grounds.

R S Pattman

Syphilis (serology and biological false-positive phenomenon)

Routine screening of hospital blood samples for syphilis. A pilot study in Mount Isa, Queensland

PM CURSON AND MN VARGHESE (Wolston Park Hospital, Wacol, Queensland, Australia). *Med J Aust* 1981;2:672.

Hydrophobic interaction chromatography used for purification of a *Treponema* Reiter ribonucleic acid antigen precipitating with antibodies in human syphilitic sera

CS PETERSEN, NS PETERSEN, AND NH AXELSEN (Statens Seruminstitut, Copenhagen, Denmark). *Anal Biochem* 1981;**117**:231-49.

Syphilis (pathology and experimental)

Avoidance of host defences by *Treponema pallidum* in situ and on extraction from infected rabbit testes

CW PENN (Department of Microbiology, University of Birmingham, UK). *J Gen Microbiol* 1981;**126**:69-75.

Ultrastructural studies on rabbit testes acutely infected with *Treponema pallidum* clearly showed that most treponemes were extracellular; although the treponemes were readily accessible to the host defence mechanisms acute inflammatory cells were rarely seen. Two lines of investigation have been used in this study to throw light on the problem of how treponemes avoid host defences in the early stages of infection.

Investigations using leucocyte migration into subcutaneous chambers were undertaken to determine whether the treponemes actively suppress or fail to incite the cellular inflammatory response. Perforated plastic chambers were implanted subcutaneously in rabbits and guinea pigs. Six to 10 weeks later the chamber fluid was inoculated with either freshly harvested live *T pallidum* or heat-killed gonococci. Leucocyte counts were made on the chamber fluids over a 48-hour period, and these showed that in the *T pallidum*-infected chambers the counts did not rise appreciably whereas after inoculation with gonococci counts increased up to 50 times the normal value. When *T pallidum* and gonococci were inoculated simultaneously a cellular response similar to that seen with the gonococci alone occurred. These results suggested that inflammation was not actively suppressed but failed to be incited by the treponemes.

The ability of treponemes to react with antibody, a necessary prerequisite for complement fixing and subsequent generation of leucocyte chemotactic factors, was also investigated. Interaction of freshly harvested treponemes with antibody from infected rabbits could not be detected by an indirect fluorescent antibody method. Lack of antibody binding to unfixed treponemes may be due to a non-antigenic barrier surrounding the treponemes, the nature of which requires further investigation.

These results taken together suggest a mechanism for the pathogenesis of early syphilis infection which revolves around the fact that the surface structure of *T pallidum* masks the organism from the host defence mechanisms.

S I Egglestone

Transfer of resistance with syphilitic immune cells: Lack of correlation with mitogenic activity

RF SCHELL, JL LEFROCK, AND JK CHAN (Hahnemann Medical College, Philadelphia, PA, USA). *Infect Immun* 1982;**35**:187-92.

Hamsters infected intradermally with *Treponema pallidum* Bosnia A developed extensive chronic skin lesions, usually accompanied by metastatic lesions on the paws, lips, and anal region and by lymph nodes teeming with treponemes. Throughout the course of syphilitic infection cells from the inguinal lymph nodes responded poorly to stimulation with suboptimal, optimal, or supraoptimal concentrations of concanavalin A, phytohaemagglutinin P, or lipopolysaccharide. The response of syphilitic spleen cells was variable. Depression of lymphocyte reactivity to mitogens preceded clinical signs of infection and correlated well with the chronicity of syphilitic infection. When syphilitic hamsters were treated with a curative dose of penicillin, their mitogenic responses returned to normal or were slightly raised. No correlation existed between mitogenic activity and the ability of lymphoid cells to induce an effective immune response when transferred to normal recipients. No significant differences in protection were detected among recipients of immune cells with or without activity to mitogens. These results show that lymphocyte transformation by mitogens in vitro is not a measure of effective treponemicidal activity and so may not be a valid indicator of the protective immune status of syphilitic animals.

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Accelerated lesion development in experimental syphilis

TJ FITZGERALD (University of Minnesota School of Medicine, Duluth, MN, USA). *Infect Immun* 1981;**34**:478-82.

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AP JOHNSON (Clinical Research Centre, Harrow, Middlesex, UK). *J Infect* 1981; **3:** 299-300.

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CR GREGG, AP JOHNSON, D TAYLOR-ROBINSON, *ET AL* (Vanderbilt University School of Medicine, Nashville, TN, USA). *Infect Immun* 1981; **34:** 1056-8.

Monoclonal antibody analysis of lipopolysaccharide from *Neisseria gonorrhoeae* and *Neisseria meningitidis*
MA APICELLA, KM BENNETT, CA HERMERATH, AND DE ROBERTS (State University of New York, Buffalo, NY, USA). *Infect Immun* 1981; **34:** 751-6.

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EG SANDSTRÖM, JS KNAPP, AND TB BUCHANAN (United States Public Health Service Hospital, Seattle, WA, USA). *Infect Immun* 1982; **35:** 229-39.

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M VIRJI (Department of Microbiology, Southampton General Hospital, Southampton, UK). *FEMS Microbiol Letters* 1981; **12:** 355-8.

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JT DOUGLAS, MD LEE, AND H NIKAIDO (Department of Microbiology, University of Hawaii, Honolulu, Hawaii, USA). *FEMS Microbiol Letters* 1981; **12:** 305-10.

Comparative in-vitro activity of cefemoxime, cefoperazone, cefuroxime, cefoxitin and penicillin against *Neisseria gonorrhoeae*
MY KHAN, Y SIDDIQUI, ML SIMPSON, AND RP GRUNINGER (University of Minnesota, Minneapolis, MN, USA). *Antimicrob Agents Chemother* 1981; **20:** 681-2.

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JA MAELAND AND SF RØE (Department of Microbiology, University of Trondheim, Norway). *Acta Pathol Microbiol Scand(B)* 1981; **89:** 335-40.

The role of outer membrane proteins in the survival of *Neisseria gonorrhoeae* Pg within guinea pig subcutaneous chambers
HM McBRIDE, PR LAMBDEN, JE HECKENS, AND PJ WATT (Department of Microbiology, Southampton General Hospital, Southampton, UK). *J Gen Microbiol* 1981; **126:** 63-8.

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AW TICE AND VL RODRIGUEZ (Clark Air Force Base, Philippines). *JAMA* 1981; **246:** 2717-8.

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JA GOLDMAN (Emory University School of Medicine, Atlanta, GA, USA). *J Rheumatol* 1981; **8:** 707-9.

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HH HANDSFIELD, VL MURPHY, AND KK HOLMES (King County Department of Public Health, Seattle, WA, USA). *Antimicrob Agents Chemother* 1981; **20:** 839-40.

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SJ LANDIS, R RAMPHAL, BJ MANSHEIM, *ET AL* (University of Florida, Gainesville, FA, USA). *Antimicrob Agents Chemother* 1981; **20:** 693-5.

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Role of chlamydiae in genitourinary disease
AW BRUCE, P CHADWICK, WS WILLETT, AND M O'SHAUGNESSY (Department of Urology, Queens University, Kingston, Ontario, Canada). *J Urol* 1981; **126:** 625-9.

***Chlamydia trachomatis* and herpes simplex virus IgA in cervical secretions of patients with cervical atypia**
K KALIMO, P TERHO, E HONKONEN, *ET AL* (University of Turku, Turku, Finland). *Br J Obstet Gynaecol* 1981; **88:** 1130-4.

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Reiter's disease
P FISK (Middlesex Hospital, London, UK). *Br Med J* 1982; **284:** 3.

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JR GREENWOOD AND K KIRK-HILLAIRE (Orange City Public Health Laboratory, Santa Ana, California, USA). *J Clin Microbiol* 1981; **14:** 699.

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A SOENDJOJO AND S PINDHA (Airlangga University, Surabaya, Indonesia). *Sex Transm Dis* 1981; **8:** 255-7.

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Adherence of *Candida albicans* to human buccal epithelial cells after growth on different carbon sources
LJ DOUGLAS, JG HOUSTON, AND J McCOURTIE (Department of Microbiology, University of Glasgow, UK). *FEMS Microbiol Letters* 1981;**72:**241-3.

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RA GREENFIELD AND JM JONES (William S Middleton Memorial Veterans Administration Hospital, Madison, WI, USA). *Infect Immun* 1981;**34:**469-77.

Immunological relatedness among *Candida albicans* and other pathogenic *Candida* species
RF HECTOR, FE LYON, AND JE DOMER (Tulane University School of Medicine, New Orleans, USA). *Infect Immun* 1981;**34:** 844-50.

The ultrastructure of *Candida albicans* infections
TJ MARRIE AND JW COSTERTON (Victoria General Hospital, Halifax, Nova Scotia, Canada). *Can J Microbiol* 1981;**27:** 1156-65.

Genital herpes

Management of genital herpes simplex virus infection occurring during pregnancy
FH BOEHM, W ESTES, PF WRIGHT, AND JF GROWDON (Vanderbilt University, Nashville, TN, USA). *Am J Obstet Gynaecol* 1981;**141:**735-40.

In a 57-month prospective study at Vanderbilt University Hospital and its affiliate, Nashville General Hospital, all suspected genital herpes lesions during pregnancy were cultured for herpes simplex virus. Those patients with positive HSV culture results before labour and without a subsequent negative culture underwent caesarean section. Those patients whose culture results became negative were considered candidates for vaginal delivery. During the study period there were 16 381 deliveries at the two institutions. Cultures were performed on 120 pregnant women; 80 were negative for HSV and 40 positive. The outcome of these pregnancies as well as a review of the experience with neonatal HSV infection in Middle Tennessee is presented. By using HSV cultures of genital lesions as a guide to determining the route of delivery, the incidence of caesarean sections and neonatal HSV infection can be kept to a minimum.

Authors' summary

Other sexually transmitted diseases

Presumed sexual transmission of meningococci
MD TALBOT AND BN COLLINS (Special Clinic and Public Health Laboratory Service, Royal Infirmary, Sheffield). *J Infect* 1981; 3:273-6.

Of three patients in whom meningococci mimicked the signs and symptoms of gonococci, one (case 1, male) had a seven-day history of urethral discharge and dysuria, one (case 2, the female extramarital consort of case 1) had no apparent abnormality in genital tract smears and cultures but harboured meningococci in her pharynx, and one (case 3, wife of case 1) had meningococci in her cervical smear and culture. In cases 1 and 3, the patients were cured satisfactorily by a single injection of 2·4 megaunits of procaine penicillin, but in case 2 the patient was not treated. All three isolates had identical biochemical reactions for meningococci and identical penicillin sensitivities. They were not groupable.

G D Morrison

Management of condylomata acuminata with the carbon dioxide laser
JW CALKINS, BJ MASTERSON, JF MAGRINA, AND CV CAPEN (Department of Obstetrics and Gynaecology, University of Kansas Medical Center, Kansas City, USA). *Obstet Gynecol* 1982;**59:**105-8.

Ninety-four patients with condylomata acuminata of the lower genital tract and perianal region were treated with the carbon dioxide laser. Most were managed in the outpatient clinic, but 1-4 treatments were required depending on the extent of the lesions. Of 90 patients, 75 (83·3%) were free of lesions on all follow-up examinations after initial treatment. Of 15 patients with recurrent lesions, 13 underwent a second laser treatment; seven of these 13 have been subsequently free of condylomata. The overall success rate was 91%. The carbon dioxide laser provides an acceptable method of treating condylomata acuminata because of its precision, rapid healing without scarring, and safety when used during pregnancy.

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Carbon dioxide laser surgery in treatment of condyloma
GA HAHN (255 S 17th St, Philadelphia, PA, USA). *Am J Obstet Gynecol* 1981; **141:**1000-10.

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MW ROSS (Department of Psychiatry, Flinders University, South Australia). *Med J Aust* 1981;**2:**670-1.

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GL CONRAD, GS KLERIS, B RUSH, AND WW DARROW (Center for Disease Control, Atlanta, GA, USA). *Sex Transm Dis* 1981;**8:**241-4.

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H YAZICI, M TUZLACI, AND S YURKAKUL (Istanbul University, Department of Medicine, Istanbul, Turkey). *Ann Rheum Dis* 1981;**40:**558-60.

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A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

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ASTM CODEN: BJVDAK 58 (4) 211-280 (1982) ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH